Gestures, Verbs, and the Motor Cortex: A TMS Study

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Abstract

Willems and Hagoort (2007) outline a number of links between language and the motor cortex. Previous researchers report the motor cortex is engaged when processing specific linguistic information such as action words, and even manual communication (e.g., gesture). Using behavioural measures, transcranial magnetic stimulation (TMS), and electroencephalographic (EEG) data, the three empirical studies reported examine the role of the motor cortex in processing both action words and gestural information.

Study One was inspired by the work of Pulvermüller, Hauk, Nikulin and Ilmoniemi (2005), who reported facilitative effects of single-pulse TMS on reaction time (RT) to action words. Pulvermüller et al. reported that RT to hand-action words decreased when TMS was applied over the hand-area of the left motor cortex at 90% of threshold and at 150 ms after the presentation of the word. The current study was designed to investigate the limits of the reported facilitative effect. Twenty-four participants (TMS: $n = 16$, control: $n = 8$) performed a word recognition task while single-pulse TMS or sham-TMS was applied at varying latencies (120 ms, 150 ms, 180 ms, 230 ms, and 290 ms) and intensities (90%, 100%, 110%, and 120%) over the hand area of the left motor cortex. RT was compared between hand-action and non-action words. Contrary to the hypothesis, no latency/intensity combinations elicited faster RT to action words and surprisingly, RT was faster to the non-action words compared to action words when TMS was applied at 150 ms/90%. This indicates that these specific TMS parameters do not necessarily specifically speed

processing for action words compared to non-action words, and it was concluded that these findings deviate from previous reports due to methodological differences.

Study Two examined the role of the motor cortex in gestural processing using a particular repetitive TMS (rTMS) protocol known as continuous theta-burst stimulation (cTBS) which has been shown to temporarily reduce motor cortex excitability (inhibition). It was anticipated that if the motor cortex is involved in gestural processing, inhibiting motor cortex function would impair processing and increase RT to gestural stimuli. cTBS was administered over the hand-area of the left motor cortex in ten participants. Participants then viewed five test stimuli words that were presented multiple times in different modalities: text, speech, gesture, and speech and gesture combined. Contrary to the hypothesis, no difference in RT between cTBS and control was found for any modality. These findings are interpreted in light of recent reports that motor cortex inhibition may not lead to behavioural change, despite being detectable in physiological excitability measures.

Study Three further investigated the role of the motor cortex in gestural processing. In addition to utilising cTBS, this study also examined EEG data, specifically event related potentials (ERP) to assist with overcoming the limitations of behavioural data. The N400 is an ERP component is widely reported to be modulated by the integration of semantically conflicting linguistic information. It was anticipated that if the motor cortex were involved in gestural processing, a cTBS-induced inhibition of the left motor cortex may cause perturbations in the N400 component. EEG data were collected from 10 participants who were presented with congruency-matching and mismatching sentence and speech-gesture stimuli. RT and N400 amplitude and latency (at central and parietal sites) were compared for these stimuli between cTBS and control. Contrary to expectations, cTBS led to

greater negativity in N400 amplitude to both stimuli types. No specific impacts on N400 amplitude to speech-gesture stimuli were found, however cTBS did have some effect on N400 latency. These results are interpreted in light of the understanding of the motor cortex as part of a network of brain areas that may be involved in cognitive processing, and the non-focal impacts of cTBS.

The findings from this series of studies are discussed with reference to the literature on the role of the motor cortex in language processing, the broader cortical impacts of TMS, and the future of research designed to examine cortical involvement in cognitive processes.

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Chapter 1: Introduction

This thesis begins with an overview of the literature connecting language and action. The theoretical framework for why language and action may be connected is rooted in the notion of embodied cognition, or the relationship between human thoughts and the actions of the body. From a neuroscientific point of view, the relationship between thought and action can be partially accounted for by an understanding of the mirror neuron system and how it functions to connect cognition with action. Mirror neurons are thought to be a subset of cortical neurons which assist with the understanding of goal-directed actions (Rizzolatti & Fabbri-Destro, 2007). Therefore, Chapter Two of this thesis contains a review of the mirror neuron literature, including their initial discovery in monkeys and the subsequent investigations into the existence of a similar mirror neuron system in the human motor cortical system. This chapter also provides some insights into how the existence of a mirror neuron system may provide a connection between language and the motor cortex, specifically relating to impact that language has on the function of cortical motor systems.

If it is accurate that humans and monkeys share a comparable mirror neuron system, an understanding of the evolutionary connection between understanding and action is also beneficial. One such connection can be seen in the gestural theory of the evolution of language (Arbib, 2002; Corballis, 2002). Chapter Three examines the notion that the origins of language lies in communicative manual hand gestures rather than vocal calls, and how a functioning mirror neuron system may have allowed early ancestors to intimate meaning from action. Focussing on the theoretical viewpoints of two principle authors Arbib (2002) and Corballis (2002), this chapter provides an explanation of what evolutionary changes needed to have

occurred for language to have emerged as well as the possible evolutionary trajectory that language took so that arbitrary meaning could be applied to abstract hand movements.

With the development of spoken and written communication, manual hand movements are no longer the primary mode of communication, save situations where it is necessary (e.g., sign language). Despite this, the vestigial remains of manual communication can still be seen in the everyday use of hand gestures that accompany speech. Chapter Four provides an overview of the literature on gesture. Specifically, this chapter reviews conceptual understandings of the purpose of gesture and its role in communication, as well as empirical evidence for the cortical representation of gesture, and how gesture may change the way the spoken communication is processed.

In many studies examining motor cortex function (e.g., Daskalakis et al., 2006; Rothwell, 1997; Wasserman, 2002), researchers report the use of a non-invasive cortical stimulation technique known as transcranial magnetic stimulation (TMS). Chapter Five provides an overview of the technical aspects of various TMS techniques, including both single-pulse and repetitive TMS (rTMS). This chapter also provides an overview of how TMS has been used to study motor cortex activity and how changes in the motor cortex may impact an individual's cognitive processes. This chapter also examines the use of TMS in language studies, and how previous authors have gained insight into cortical linguistic functions through the application of TMS.

One specific link between motor cortex and language is the role that the motor cortex may have in processing *action-related* language. Chapter Six presents

an empirical study that investigates the impact of single-pulse TMS over the hand area of the motor cortex on processing action-related language. The impacts of TMS on motor-language have been previously investigated and it has been reported that a TMS pulse delivered over the hand area of the motor cortex at a specific intensity and latency following the presentation of hand-related action word can lead to faster reaction time (RT) to those words (Pulvermüller et al., 2005). The study outlined in this chapter further tests this effect by manipulating pulse intensity and latency and investigating the effects of TMS on RT to hand action words compared to non-action words. The results are presented and interpreted within the framework of the single-pulse TMS literature.

As identified in the literature review chapters, another link between language and action is provided in the form of gestural communication. Based on the notion that gestures are observable actions that carry meaning, they would theoretically activate mirror systems, and previous empirical researchers have reported that when processing gestural information, increased activity is observed throughout the cortical motor systems, including the primary motor cortex (Skipper, Goldin-Meadow, Nusbaum, & Small, 2007). Chapter Seven presents an empirical study that investigates the involvement of the motor cortex in gestural processing. After applying continuous theta-burst stimulation (cTBS) over the motor cortex to create a 'virtual lesion' (temporary reduction in cortical excitability), participants were required to identify a selection of words presented in four different modalities: text, speech, gesture, combined speech and gesture. RT data with and without the application of cTBS are examined and the results are discussed.

In addition to behavioural measures of gestural processing, other techniques such as electroencephalography (EEG) can provide complementary data that assists

with understanding the underlying neural processes. Sections of recorded EEG data, known as event-related potentials (ERPs) can be sampled as unique waveforms that are time-locked to the presentation of a specific stimulus. The N400 is a distinct negative going peak in the ERP waveform that has been found to be modulated when an individual attempts to integrate mismatching semantic information. Chapter Eight details an empirical study that examines changes in the N400 component in participants who are presented with gestural information that is incongruent with speech information. This study also adopts the 'virtual-lesion' approach to inhibit motor cortex excitability. In turn, this allows for the observation of changes in the N400 component which may be attributed to cTBS over the motor cortex. Results from behavioural and electrophysiological recordings are reported, and an interpretation of these findings is provided.

The final chapter of this thesis provides an overview of the results of all three empirical studies and despite the methodological differences between these studies reported, this chapter offers an interpretation of how these results fit within theoretical frameworks and the existing literature. This chapter also provides suggestions for further research directions which may assist in providing answers to the new questions generated through this research.

Chapter 2: Mirror Neurons

The aim of this chapter is to provide an overview of the literature related to the current understanding of the mirror neuron system. This chapter covers information regarding the initial discovery of mirror neurons in the F5 cortical area in the brains of monkeys, and the neuroimaging evidence that indicates the existence of a similar system in the human brain. Additionally, this chapter offers greater insight into the functional role of the mirror neuron system and how it relates to cortical systems responsible for manual action as well as the production and comprehension of language.

It is now recognised that mirror neurons do not function by themselves but operate as part of a larger extensive network involving other cortical structures including the superior temporal sulcus (STS) and the inferior parietal lobule (Rizzolatti, Fogassi, & Gallese, 2001). Corballis (2010) suggests that the homologous areas of the human brain are also involved in this action-understanding network. For example, human brain imaging studies have demonstrated that the superior temporal cortex (homologous to STS) is thought to be involved in decoding complex social signals from an observed action (Puce & Perrett, 2003).

History of Mirror Neurons

In 1988, Rizzolatti and colleagues published a research paper outlining the discovery of a selection of neurons in the F5 area of the frontal lobe of macaque monkeys. From these initial data, Gallese, Fadiga, Fogassi, and Rizzolatti (1996) reported that a newly discovered set of neurons known as 'mirror neurons' became active when the monkey performed a given action or observed a similar action performed by the experimenter. They went further to say that these neurons were only triggered upon sighting the interaction of the human action with the target

object (e.g., a hand grasping an apple) and were not triggered by observation of the object or experimenter alone. Gallese et al. also report that the hand and mouth actions were the most effective at eliciting responses, and the actions that were observed to be the most represented amongst the activating mirror neurons were actions of grasping, manipulating, and placing. It was concluded that mirror neurons form a system which matches the observation and execution of motor actions. Gallese et al. further found that despite there being activity throughout the mirror neuron system during action observation, there are groups of mirror neurons that are activated by the observation of one action only (such as grasping, or manipulating an object) and then reduce or stop activity when the action is completed. Gallese et al. provide an example in which they recorded neural activity in a set of mirror neurons in response to the observation of a grasping motion. Following the completion of the grasp motion, many of these neurons reduce in activity and then if the object is manipulated, neural activity increases in a notably distinct set of mirror neurons. Initially, researchers reported that action imitation was the primary role of the mirror neuron system, as research on the human brain indicated that these systems were activated as part of an observation and imitation sequence (Corballis, 2010). More recently, many researchers tend to agree that mirror neurons do not serve a simple imitation role in monkeys, but that they actually operate as a part of a system of action *understanding*. This means that while mirror neurons assist the monkey (or human) to understand the actions performed by others by mapping observed behaviour to actions that it can perform itself, it does not mediate the actual performance of the motor patterns required for imitation (Corballis, 2010).

Results from a series of studies have allowed researchers to conclude that the mirror neuron system has a more complex role than simply assisting in the

observation and execution of motor actions, and instead is thought to have a role in *understanding* action. In one study it was tested whether mirror neurons would respond to an action merely based on the sound alone (Kohler et al., 2002). Mirror neuron activity was initially recorded while monkeys observed a motor act such as breaking a peanut shell or ripping paper. Remarkably, many of these same neurons also triggered when the monkeys heard only the sounds of these actions, leading the researchers to surmise that some mirror neurons are both aurally and visually triggered.

In a second series of experiments, researchers investigated the impact of further hindering a monkey's ability to observe an action sequence (Umiltà et al., 2001) . In this study, monkeys initially observed a complete action sequence (such as grasping) and mirror neuron activity was recorded. Interestingly, when researchers occluded the final phase of the movement sequence (e.g., visually hiding the grasping hand moments before reaching its intended target), they still reported activity in a substantial subset of F5 mirror neurons. These results were further refined in another two conditions in which the action was mimed, with one complete mimed sequence and another partially obscured mimed sequence. The researchers report that neither of the mimed conditions evoked mirror neuron activity, and they concluded that the only difference between the two obscured conditions (mimed or real) was the monkey's 'knowledge' of the object still existing even though it was hidden. They therefore suggest that it is not just the basic manual action that triggers mirror neurons, but the fact that the action is part of an intention-based goal-directed action sequence. The results from both of these studies demonstrate that mirror neurons can operate in absence of, or in extension to, what is actually observed which supports the notion that mirror neurons are not simply part of an action

observation system, and that they actually underpin a cortical system of action understanding (Rizzolatti & Fabri-Destro, 2007).

Experimental Evidence for a Mirror Neuron System

Since their initial discovery, many researchers have sought to better understand the nature and function of mirror neurons, and how they connect and interact with surrounding cortical structures. A selection of these papers is reviewed in this section.

Early mirror neuron research largely used data gathered from electrodes placed directly into the brains of monkeys (e.g., Gallese et al., 1996). Due to the obvious ethical constraints of recording electrical activity directly from neurons in the human cortex, researchers must rely on other recording and imaging techniques when seeking evidence for the existence of a mirror neuron system in humans. A functional magnetic resonance imaging (fMRI) study conducted by Buccino and colleagues (2004) investigated the cortical areas which were active during the observation of mouth actions. Human subjects observed silent video recordings of dogs, monkeys, and humans biting and performing communicative actions (e.g., barking, lip smacking, and speech respectively). The observation of speech activated the pars opercularis of the inferior frontal gyrus in the left hemisphere (part of Broca's area), the observation of lip-smacking activated a small focal point of the pars opercularis bilaterally, and the observation of barking did not produce any activation in the frontal lobe. It was also noted that observation of all types of mouth actions induced activation in the extrastriate occipital areas. The authors proposed that as the activation of pre-motor areas was only seen when participants were observing speech and lipsmacking, only actions belonging to the motor repertoire of

the observer are mapped on his/her motor system and actions that do not belong to this repertoire appear to be processed solely on their visual properties.

Further evidence is provided by Muthukumaraswamy and Johnson (2004) who examined the changes in EEG recordings taken from the area of scalp over the sensorimotor cortex. At rest, the sensorimotor cortex produces a regular EEG pattern of approximately 8 to 13Hz known as the rolandic mu rhythm (Muthukumaraswamy & Johnson, 2004). Previous studies have shown that the mu rhythm is diminished or extinguished when the motor cortex is activated during the execution of movement and also desynchronises when subjects are observing but not executing movement (Cochin, Barthelemy, Roux, & Martineau, 1999). Muthukumaraswamy and Johnson further report that a lower amplitude desynchronised mu rhythm was recorded across the sensorimotor cortex when participants observed a goal-directed hand action (grasping an object), but not when observing a simple non-interactive hand extension. It is suggested that the changes in mu rhythm may reflect processes that are occurring in the mirror neuron system which incorporates cortical areas surrounding the rolandic fissure. The finding that changes in mu rhythm were detected when subjects performed a goal oriented movement also supports the notion that the mirror neuron system is not a system for merely observing actions, but may actually be a system which facilitates understanding of action.

To further investigate the role of mirror neurons in understanding actions, Iacoboni and colleagues (2005) conducted an fMRI study looking at mirror neuron function where participants watched video clips of simple motor actions in which the intention of the performer was manipulated. More specifically, participants observed video-clips that conformed to one of three different conditions. In the "context" condition, participants saw a mug or plate set out with cakes and tea to either look

like someone was preparing to eat or drink, or had just finished. In the "action" condition, participants saw a performer reach and grasp a mug or a plate, however there was no food or drink so the action was context-free. In the final "intention" condition, the action is embedded in context and the video-clip showed the performer picking up the mug or plate with the intention of consuming food or drink or cleaning up the used crockery (depending on the state of the cup or plate). The basic assumption was that the context provided by this initial image would assist the viewer to determine whether the cup would be picked up in such a way as to be consumed, or picked up to be washed. These context-embedded actions were compared against action-only clips, and context-only clips. Iacoboni et al. report different patterns of activation in suspected mirror neuron areas that are unique to the context-embedded action clips, and were not seen in the other conditions. More specifically, increased activity was seen in the posterior part of the inferior frontal gyrus and the adjacent area of the ventral premotor cortex. The authors concluded that premotor mirror neuron areas previously thought to only be involved in action recognition are also involved in understanding the intent of others' actions (Iacoboni et al., 2005; Rizzolatti & Fabbri-Destro, 2007).

A series of positron emission tomography (PET) studies have provided evidence to support the cerebral location of the mirror neuron system. A PET study by Grafton, Arbib, Fadiga, and Rizzolatti (1996) and a related PET study conducted by Rizzolatti et al. (1996) shared a condition in which participants observed the experimenter grasping an object. When compared with a control condition (inanimate object observation), it is reported in both studies that during the observation of a grasping action there is activity in the left inferior STS (Brodmann's area 21), the anterior part of Broca's area (Brodmann's area 45), the left rostral

inferior parietal cortex (Brodmann's area 40), the rostral part of the supplementary motor area, and the right dorsal premotor cortex. Grafton et al. and Rizzolatti et al. proposed that these areas may form part of a circuit for the recognition of hand-object interactions.

TMS is another technique used to provide evidence supportive of the existence of a mirror neuron system in humans. Maeda, Kleiner-Fisman, and Pascual-Leone (2002) conducted a study where they measured the change in the excitation of cortical areas responsible for finger and thumb movements while participants watched video clips of these movements. In this study, specific TMS-induced electromyographic (EMG) recordings known as motor evoked potentials (MEPs) were taken from the muscles on the thumb and forefinger. Increases or decreases in the amplitude of the MEPs were noted to be representative of changes in excitability of the cortical areas being targeted by TMS. Maeda and colleagues reported that when participants observed finger or thumb movements there was an increase in excitability of the cortical pathways responsible for controlling the movement of that particular muscle group. Furthermore, they also reported that the degree of modulation in excitability is specific to the orientation of the hand observed on the video clips. They found that a greater increase in excitability is recorded when the hand in the video clip more closely matches that of the observer, and reduced excitability when the hand in the video is rotated 180 degrees to appear opposite to that of the observer. Maeda and colleagues suggest that while their results may initially seem contradictory to mirror neuron theory (as it would be expected that observation of 'other's hand' would be more likely to activate a mirror neuron system than observation of 'self's hand'), previous research on mirror neurons utilised mirror-image action observation (which is much more

environmentally realistic), rather than rotated-image action observation. Results such as these provide supporting evidence for the existence of a mirror system in humans (Fadiga, Craighero, & Olivier, 2005).

Further evidence supporting the existence of a mirror neuron system in humans is reported in another TMS study conducted Gangitano, Mottaghy and Pascual-Leone (2004). In that study, participants observed a video-clip that showed a normal reach and grasp motion, or an anomalous reach and grasp motion that began the same at the normal motion but also included a delayed grasp opening motion or unexpected early grasp closure. Using MEP recordings from different time points throughout the video-clip, Gangitano and colleagues were able to map a profile of cortical excitability to the kinematic profile action in the video-clip. It was reported that observation of the anomalous movements either did not evoke any modulation of cortical excitability or evoked a profile similar to that of the common grasp and reach movement. The authors conclude that not only does this provide evidence for a neural system which develops motor plans based on observation (the mirror neuron system), but that this motor plan is developed at the beginning of an observed movement and tends to proceed to completion despite changes to visual cues.

Mirror Neurons Beyond Action Imitation

The evidence presented so far indicates that the mirror neuron system is primarily involved in the understanding (and 'covert rehearsal') of action, allowing monkeys and humans to imitate and comprehend the actions of others. However, throughout the last decade, researchers have credited mirror neurons with involvement in an array of cognitive functions beyond that of simple action understanding, including theory of mind, empathy, and language. The role of mirror

neurons in language is a rapidly expanding field of research and is one of the fundamental theoretical bases for this thesis, therefore it shall be more adequately explored later in this chapter, and this section shall focus on other suggested roles of mirror neurons.

Some researchers (e.g., Gallese & Goldman, 1998; Oberman et al., 2005) suggest that the mirror neuron system plays a crucial role in an individual's ability to develop a fully-functioning theory of mind allowing them to see the world from another person's point of view and accurately empathise with others. From a purely theoretical basis, if mirror neurons allowed not only observation of action, but also assisted the observer to understand the action, then the observer may be able to estimate the goals or intention of others based on their actions. Gallese and Goldman suggest that if the observer can draw insight into the purpose and motivations for the action of another, this provides a basis for the development of a theory of mind.

Through their research into Autism Spectrum Disorders (ASD) Oberman et al. (2005) provide some empirical evidence to support the role of the mirror neurons system in the development of theory of mind and empathy. ASD are a range of disorders largely characterised by deficits in language, imitation (facial expressions and movement), theory of mind and empathy, as well as an array of behavioural and communicative deficits and sensory sensitivities (Sadock, 2007). An EEG study conducted by Oberman et al. reported that the mirror neuron systems is not functioning correctly in individuals with ASD. Specifically, Oberman et al. investigated difference in mu rhythm changes between a group of ASD subjects and a control group. As mentioned earlier it has been suggested that suppressions and desynchronisation of the mu rhythm in specific cortical areas during action observation as well as action execution is evidence of a functional mirror neuron

system (Cochin et al. 1998; Muthukumaraswamy & Johnson, 2004). Oberman et al. required participants to watch a video of a moving hand, a bouncing ball, or white noise, or required them to move their own hand. The researchers recorded a suppression of the mu rhythm in control participants when they moved their own hand and observed a video of a hand moving. This mu suppression was not observed in the ASD participants leading the researchers to conclude that the mirror neuron system (which would usually be engaged by this task) is dysfunctional in those with ASD.

In another study investigating mu rhythm attenuation in an ASD sample, Bernier, Dawson, Webb and Murias (2007) recorded EEG data when ASD and control participants were required to observe an action, perform an action, and imitate another performing an action. Similar to Oberman et al. (2005), Bernier et al. reported the mu rhythm of ASD subjects was less attenuated in the observation condition compared to controls, however unlike Oberman et al., Bernier et al. found both groups demonstrated a significant attenuation of the mu rhythm when executing an action. Furthermore, Bernier et al. also found that in the ASD group low levels of attenuation were positively correlated with behavioural measures of performance in the imitation task. The researchers therefore infer that a more responsive mu rhythm (indicative of a functioning mirror neuron system) is directly related to an individual's ability to imitate an action (Bernier et al., 2007). In a follow-up article, Oberman and Ramachandran (2007) provide an in-depth evaluation of the relationship between the mirror neuron system and ASD. They conclude that while other cortical 'simulator systems' are involved in the regulation of many symptoms of ASD, they believe that the mirror neuron system is the "unifying mechanism that underlies deficits in imitation, theory of mind, empathy and pragmatic language"

(Oberman & Ramachandran, 2007, p. 322). While these studies do not provide incontrovertible evidence of the existence of a human mirror neuron system, the conclusions proposed by these authors do provide some insight into the potential role that the mirror neuron system may have in enhancing an individual's ability to interact with their environment.

Gallese and Lakoff (2005) discuss the role of the mirror neuron system in the broader scientific and philosophical understanding of how thoughts are processed by the brain. Specifically, Gallese and Lakoff outlined the notion of embodied thought, or embodied cognition. That is, there is a common neural substrate responsible for both imagining and performing an action. Gallese and Lakoff propose that the sensory and motor systems of the brain do not operate in a modular fashion, with some cortical areas independently dealing in conveyance/processing of abstract concepts (thoughts) while other cortical areas are responsible for moving limbs to interact with the environment. Instead, the sensory-motor system is thought to be multimodal, an adaptation or "neural exploitation" (p. 456) that allows this network to perform more advanced functions than those of its individual parts. Gallese and Lakoff highlight the mirror neuron system as the fundamental link between associated motor and sensory functions, allowing this system in human cognition to engage in more advanced functions such as language.

While the proximity between language processing and the mirror neuron system is not initially clear from Gallese and Lakoff's review(2005), they do provide some rationale for why they think the mirror neuron system (as part of the sensory-motor system they describe) has a role to play in language. Firstly they argue that the sensory-motor system is not just responsible for action or for observation, but that the system is able to "characterise a sensory-motor concept" (p.468). Gallese and

Lakoff express that this is not limited to action concepts, but to all concrete concepts. They give an example of a chair and how we are able to observe a chair, imagine observing a chair, and also imagine what it would be like to feel or sit in that chair. While it could be argued that the engagement of the sensory-motor system may be reasonably salient in this chair example, it is less clear how the sensory-motor system is involved with some other concrete concepts (e.g., a 'comet'), which have a much less distinguishable link with the human body and the way it interacts with the concrete object being conceptualised.

A more reserved proposal for the role of the mirror neuron system in language processing is outlined by Fischer and Zwaan (2008), who specify that the notion of 'embodied language' largely only applies to language that is related to action. Fischer and Zwaan cite a number studies that show merely hearing language about an action can disturb the execution of subsequent motor movements. For example, Glenberg and Kaschak (2002) asked participants to move their hand from a central button to one of two buttons that were positioned either closer to, or further from their body based on sensibility judgment of a presented sentence. An example sentence is "close the drawer", a description of an action that is usually executed by moving the hand away from the body. Glenberg and Kaschak measured the amount of time it took participants to make a decision and release the centre button. Participants found it more difficult to respond when their response required them to move their hand to the button located in an area proximally mismatching the action described in the sentence. In the 'drawer' example, button release times were delayed when responding required the hand to move to the button closest to them, indicating a conflict between the response action and the action described in the sentence. Results from studies like the above led Fischer and Zwaan to conclude that there is

an interaction between language and motor systems, and that motor related language is embodied by engagement motor systems, rather than just existing as an abstract concept. More insights into the interactions between mirror neurons and language are provided below.

Mirror Neurons and Language

Speech

As mentioned previously, mirror neurons were initially discovered in the F5 area of the monkey frontal cortex. This area is homologous to the region of the human brain known as Broca's area, which is an area of the brain heavily involved in language production and some language comprehension tasks. More precisely Broca's area can be divided into Brodman's areas 44 and 45. Area 45 is activated by both spoken and signed language output (Horowitz et al., 2003), whereas Area 44 (the true analogue of F5) is activated by non-linguistic motor functions including complex hand movements, and sensorimotor learning and integration (Binkofski & Buccino, 2004). It is not only language production areas of the cortex that appear to be linked with mirror neuron networks. As mentioned above, the role of STS, which is involved in the action understanding network in monkeys is homologous to Wernicke's area in the human brain – an area responsible for a vast array of complex language comprehension tasks such as the identification of phonetic information in a speech stream (Boatman, 2004; Scott, Blank, Rosen & Wise, 2000).

The links between language and the mirror neuron system can be seen in the most basic units of speech, right through to the more complex semantic content of a word. As is reviewed above, there is an abundance of evidence to support the notion of a mirror neuron system that responds almost entirely to stimuli that are presented

visually to an individual. This is largely due to most researchers demonstrating mirror neuron activity occurs primarily in response to the observation of an action. However, as mentioned above, some researchers have found that the mirror neuron system also responds to the sounds of actions. Kohler et al. (2002) gave an example of this when they observed mirror neuron activity in a monkey cortex in response to the sound of tearing paper.

When understanding this aural activation of mirror neurons, it is important to review some of the literature surrounding the motor theory of speech perception (Liberman & Mattingly, 1985), or the updated theory of articulatory phonology (Browman & Goldstein, 1995). The basic premise of articulatory phonology is that speech is perceived, at least partially, as a series of oral muscle movements or 'gestures'. In other words, each of the familiar vocal sounds that humans perceive can be understood as a series of gestures made by articulatory organs in the mouth and throat; the lips, the velum, the larynx, and the blade, body and root of the tongue (Corballis, 2010; Goldstein, Byrd, & Saltzman, 2006). Although it is clear that the ability to understand speech goes beyond simple aural sound processing, Gentilucci and Corballis (2006) point out that we can perceive speech at up to 10 to 15 phonemes (smallest unit of meaning sound) per second, a rate at which we are unable to effectively process simple sound units such as tones or noises. Gentilucci and Corballis go on to suggest that the complexities of speech perception give rise to the possibility that articulatory phonology can account for at least part of our speech recognition ability. Hickok (2009) argues against the motor theory of speech perception and the role of mirror neurons. He suggests that this theory does not account for individuals with Broca's aphasia who are largely still able to comprehend speech, even though they struggle to produce it. Hickok reports that individuals with

Broca's aphasia often have lesions throughout the surrounding area of the brain including the lateral front lobe, the motor cortex, and the anterior insula, and that lesions like this effectively damage the entire left hemisphere mirror system. Hickok reasons that if the motor theory of speech perception were true, Broca's aphasics would be unable to comprehend language, however Hickok may be overlooking the other non-lesioned parts of the brain (such as Wernicke's area) which also process aural speech information (Boatman, 2004).

Although the notions of articulatory phonology or motor speech perception cannot be unequivocally attributed to the operation of mirror neurons, there is empirical evidence to support the notion that speech processing involves areas of the motor cortex and that some 'covert rehearsal' of the motor programs needed for speech production occur when perceiving speech. In one study, Wilson, Saygin, Sereno and Iacoboni (2004) collected fMRI data from participants while requiring them to produce or listen to a selection of monosyllabic speech sounds. As expected there was bilateral fMRI activity in the speech production areas of the motor cortex when participants produced the sounds. Interestingly, there was also bilateral activity recorded in the ventral premotor cortex that largely overlapped with the speech production area. Activity in these areas may be indicative of speech production motor systems operating during speech perception (Wilson et al., 2004). In a related fMRI study, participants listened to both speech sounds that were native to their language as well as non-native speech sounds (Wilson & Iacoboni, 2006). Compared to native speech sounds, non-native speech sounds are those that do not usually occur in the native language. An example of this would be the phoneme /l/ (as in *luck*), which occurs in English, but does not occur in Japanese. The researchers found that there was increased activity in the speech production area of the motor

cortex when listening to non-native speech sounds, which they attribute to the notion that the motor system can only simulate known phonemes, and perceiving an unknown phoneme engages the motor system in repeated attempts to find a matching model (Wilson & Iacoboni, 2006). Regardless of the validity of the explanation for the increase in activity, the more salient observation is that the motor regions of the brain are sensitive to the familiarity of the perceived speech sounds, providing more evidence that the motor system plays an active role in speech perception.

To investigate the effect of a virtual lesion over the premotor cortex, Meister, Wilson, Deblieck, Wu, and Iacoboni (2007) used rTMS to temporarily impair the function of the left premotor cortex while participants were required to perform a simple speech sound discrimination task, a colour discrimination task, or a tone discrimination task. The researchers reported that rTMS over the motor cortex impaired accuracy on the speech discrimination task significantly more than that of the other two tasks, implying that the premotor cortex processes information specific to the perception of speech sounds. While not suggesting that the motor system is critical to speech perception, studies such as those by Wilson et al. (2004), Wilson and Iacoboni (2006), and Meister et al. demonstrate that it certainly plays a role. Furthermore, the results of these studies concur with mirror neuron theory in that they suggest that motor patterns - in this case the configuration of lips, tongue, etc. that produce speech - are rehearsed within the motor cortical areas when listening to speech, an extension of the findings of Kohler et al (2002) .

Motor-Related Language

Beyond the theories of motor speech perception or articulatory phonology, there is also a growing body of evidence to support the notion that the motor cortex

may have a greater role in processing words that are related to actions. In other words, there is evidence to suggest that mirror neurons are involved in linguistic tasks with a motor component, rather than simply motor tasks with a linguistic component.

A number of fMRI studies conducted over the past decade have yielded results demonstrating that the premotor and motor cortices are involved in processing action-related language. Hauk, Johnsrude and Pulvermüller (2004) found that there were overlapping activation patterns within the motor cortex when participants read simple action verbs (such as *pick* or *kick*), similar to when they actually performed the action with the associated effector, in this case as the hand or foot. The authors cite this as evidence that there are similar cortical areas activated by the two different tasks, which concurs with the notion that they are part of a system that involves both action and language components. Aziz-Zadeh, Wilson, Rizzolatti and Iacoboni (2006) conducted an fMRI study in which participants read phrases that described actions of different effectors (i.e., hand, foot, or mouth), or observed someone performing these actions. They observed an increase in cortical activity in effector-specific areas of the left premotor cortex when observing actions or listening to action phrases that involve that effector (Aziz-Zadeh et al., 2006). Aziz-Zadeh et al. interpret these findings as evidence that it is the semantic component of the motor language that determines the recruitment of specific areas of mirror neurons within the premotor cortex.

In a similar fMRI study, Tettamanti et al. (2005) yielded results that support the notion that the motor cortex is involved in processing action related sentences. In this study, participants listened to sentences describing actions performed by the mouth, the hand, or the foot (e.g., "*I bite an apple*", "*I grasp a knife*", and "*I kick the*

ball"), or sentences with abstract content "*I appreciate sincerity*". The fMRI results showed that Broca's area was the only cortical region active across all action conditions compared to baseline. Tettamanti et al. also found that there were activity increases at many sites including the inferior parietal lobule, intraparietal sulcus, posterior middle temporal gyrus, as well as sections of the premotor cortex responsible for the motor patterns the action sentence was related to. It is important to note that the activation of motor systems by action-related words is not limited to action verbs and may also extend to action-related nouns (such as ballerina or screwdriver). In a PET study, Vigliocco et al. (2006) found that there was an increase in motor cortex activity when comparing action words (both verbs and nouns) to sensory words (also both verbs and nouns).

In contrast to the observational nature of fMRI or PET studies, Pulvermüller et al. (2005) used TMS to investigate the impact of stimulating motor cortex activity while processing action words. In this study, participants were to respond to a series of action words that were either hand actions (e.g., *pick*) or leg actions (e.g., *kick*) while undergoing TMS of the hand or leg area of the left motor cortex. In the study, as TMS had a facilitatory effect on the targeted region of the motor cortex, Pulvermüller at al. found that when TMS was applied to the hand area of the motor cortex participants responded faster to hand action words than leg action words, and TMS to the leg area of the motor cortex facilitated faster reaction times to leg action words. Similar to the conclusions of the authors of the above fMRI studies Pulvermüller et al. regard this as direct evidence that not only is the motor cortex involved in processing action words, but that effector (arm or leg) specific words are processed by the effector specific area of the motor cortex. In summary, these studies demonstrate that cortical systems for processing action words or sentences

describing an action overlap with those that perform the related action, akin to earlier research which demonstrated that observation of action also stimulates associated motor circuits as well as Broca's area (Grafton et al. 1996: Rizzolatti et al. 1996). Therefore, these studies also provide sound evidence for the incorporation of linguistic stimulation as an activator of the mirror neuron system.

While the above studies draw their inferences from cortical activity (either in the form of fMRI or TMS-induced changes in EMG), Zwaan and Taylor (2006) designed a series of experiments which examined the behavioural impact of language on motor performance (see also Taylor & Zwaan, 2008). Citing mirror neuron research as the primary basis for their study, Zwaan and Taylor use the term "motor resonance" to describe the neural activity seen in the motor cortex and surrounding areas when observing someone else performing an action. In the first study, participants performed a manual rotation task (turning a knob with their hands) while observing a stimulus that was either rotating in a congruent or incongruent direction. Faster reaction times were recorded when observing the congruent stimulus, which the researchers interpreted as visual information leading to stimulation of the mirror neurons and ultimately facilitation of motor cortex function. Furthermore, they also found that reaction times were faster when participants heard congruent audio descriptions of the task ("screw"/"unscrew"), which Zwaan and Taylor interpret as a possible linguistic activation of mirror neuron circuitry which creates 'motor resonance' in a similar fashion to the observation of action.

To further explore the findings of this first study, Taylor and Zwaan (2008) had participants rotate a knob while reading sentences that contained a turning action (e.g., "the athlete was given a bottle of water which he opened quickly"). They report

that participants' reading speed slowed when rotating the knob in a direction incongruent to that of the stimulus sentence. Furthermore, they found that the impact on reading speed persisted when the adverb at the end of the sentence was related to the motor action (e.g., opening the bottle 'slowly' or 'quickly') as opposed to the relating to the protagonist (e.g., 'happily' or 'eagerly'). They also report that it is the incongruent verb specifically (and its associated adverb) which impacts speed of reading, as the reading speed of the other words in the sentence was not affected by the performance of a simultaneous motor action (i.e., knob rotation). Taylor and Zwaan conclude that this is evidence of the impact of language on motor cortex activity, and results from both of these behavioural studies (Zwaan & Taylor, 2006, and Taylor & Zwaan 2008) may be taken to indicate that 'motor resonance' is not just limited to the impact of action observation on language perception or action performance, but may reflect a bidirectional influence between language perception and action performance.

As has been demonstrated earlier in this chapter, mirror neurons have been attributed with involvement in a number of cortical networks and cognitive abilities, and the argument for mirror neuron involvement in speech perception via articulatory phonology theory is easily explainable as it falls largely under the umbrella of 'action observation'. Contrastingly, understanding the exact role of mirror neurons in processing action-related language requires more careful consideration. The above research lends itself to a number of conclusions; motor associated language is processed by cortical motor networks and effector-specific cortical areas, it triggers activity in the motor networks similar to action observation or action performance, and motor activity and stimulation of the motor cortex influences language processing ability. In summary, the simplest explanation for

these findings is that processing action-related language activates the mirror neuron system which incorporates areas such as the motor and premotor cortices, and sufficient manipulation of any component of this system will impact processing/performance in other parts.

Chapter 3: Evolution of Language

"*I once tried to explain the gestural theory of language to a prominent linguist, who dismissed it with an eloquent wave of his hand.*"

- Corballis, 2002, p.100

Advances in understanding the mirror neuron system provides evidence of the link between meaning conveyed through language and the parts of the brain responsible for action (such as the motor-cortex) (e.g., Pulvermüller et al., 2005; Zwaan & Taylor, 2006). When examining how the mirror neuron system is linked with language, it is fundamentally important to understand *why* this link between language and action exists. Tied in with observations of hemispheric linguistic specialisation and the prevalence of right-hand dominance (explored in more detail below), the gestural theory for the evolution of language goes some way to explain the evolutionary function of the mirror neuron system and the links between language and action. It is tempting to assume that because speech is the primary mode of modern human communication, then this was always the case (e.g., Hauser, Chomsky & Fitch, 2002), however opponents to the speech-initiated theory of the evolution of language believe that modern language began in the form of gestural communication rather than speech (Corballis, 2002).

While it is very difficult (if not impossible) to irrefutably establish whether the origins of language were gestural or spoken, it is still important to evaluate the available evidence and examine theories of the evolution of language. A leading theorist admits that as the record of human writing only goes back approximately

6000 years, theorising on the linguistic skills of early hominids needs to be seen as an "hypothesis that is based on, but in no sense implied by, a variety of evidence" (Arbib, 2002, p.27). The literature abounds with controversy on whether language first evolved as speech or gesture. In fact, Fitch (2000) indicates that the historical controversy surrounding the evolution of language was so fierce that in 1886, discussion of the topic was banned by the Linguistic Society of Paris. As this thesis focuses on the relationship between action and language, this chapter is by no means designed to resolve this controversy, nor to present equally-weighted cases from both sides. Instead, this chapter provides a review of the work of two primary proponents of the gestural theory of language and the evidence used to support their claims, while providing a brief overview of speech-initiated evolutionary language theories.

Language within the Cortex

Before examining the literature on how humans gained linguistic ability, it is important to review the current scientific understanding of how language is processed in the brain. For most people, the majority of language is processed within the left hemisphere of the cerebral cortex. Two specific cortical regions, Broca's and Wernicke's areas and the inter-connecting pathways are attributed with a range of linguistic tasks which can broadly be classified as language production and language comprehension.

Broca's Area

Broca’s area is located in the inferior frontal gyrus of the left hemisphere, towards the anterior end of the arcuate fasciculus (a bundle of axons which connects Broca's area with Wernicke’s area), and it is generally regarded as encompassing the

pars opercularis and pars triangularis in this region (or Brodmann's areas 44 and 45) (Dronkers, Plaisant, Iba-Zizen & Cabanis, 2007). Named after Paul Broca who in 1861 observed that patients with damage to this part of the brain experienced expressive language disorders (e.g., aphasia), this cortical area is traditionally thought to be the primary section of the cerebrum responsible for language production. Although modern imaging of the preserved brains of Broca's original patients shows that the lesions extend beyond this area (Dronkers et al., 2007), the acceptance of the role of Broca's area in language production still persists. Broca's area is responsible for the formation of language and appropriate motor pattern that would be used to communicate language. While Broca's area does not directly communicate with the muscles used for speech, it does project motor information bilaterally to the motor cortex, which is positioned on the posterior end of the frontal lobe along the Rolandic fissure. The motor cortex then executes the motor pattern provided by Broca's area by activating neurons responsible for muscle groups that control speech (such as the tongue, lips, jaw, cheeks, larynx, and diaphragm) (Rosenzweig, Breedlove, & Leiman, 2002).

Some authors, such as Indefrey and Levelt (2004) suggest that the traditional view of Broca’s area is outdated, and that it is an oversimplification to see this area as the sole centre for language production. Although it is accepted that Broca’s area has a primary role in language production, there is evidence indicating that it is also involved in aspects of receptive language processing. Citing evidence from fMRI and TMS studies, Skipper et al. (2007) suggest that Broca’s area is highly involved in phonetic processing as well as processing semantic content of linguistic information. Skipper et al. hypothesise that as Broca’s area contains neuronal pathways ultimately responsible for motor sequences that (via the motor cortex)

control the complex movement of speech efforts (tongue, lips, larynx, etc.) during speech. The involvement of Broca's area in phonetic processing is thought to be to due to the engagement of these neurons during the observation of others speaking (Skipper et al., 2007).

Although there is no doubt that Broca's area is highly involved in language production, it is likely that there is a larger network of cortical areas responsible for specific language production functions. In a meta-analytic review, Indefrey and Levelt (2004) summarise the current literature on the cortical regions linked with language production. The authors identify a number of different areas involved in word production including four right-hemispheric regions (the mid superior temporal gyrus (STG), the supplementary motor area (SMA), and medial and lateral cerebellum) and 11 left-hemispheric regions (posterior inferior frontal gyrus, ventral precentral gyrus, SMA, mid and posterior superior and middle temporal gyri, posterior temporal fusiform gyrus, anterior insula, thalamus, and medial cerebellum).

Indefrey and Levelt (2004) also offer details of the specific language production roles that each of these cortical regions performs. Lexical (word) selection is believed to occur in the mid-section of the left middle temporal gyrus. Retrieval of phonological information for word formation activates the right SMA, the left anterior insula, and the left posterior, superior and middle temporal gyri (Wernicke's area). Syllabification (dividing words into their syllabic components) and stored lexical knowledge is largely the responsibility of the left posterior inferior frontal gyrus (Broca's area). Indefrey and Levelt note that the final steps in language production, phonetic encoding and articulation, are the most difficult to confine to a specific cortical region. In total, the authors have described 17 cortical regions thought to be involved in phonetic encoding and articulation, 12 of which are part of

the central nervous motor systems (bilateral ventral motor and sensory regions, right dorsal motor region, right supplementary motor area, left and medial right cerebellum, bilateral thalami, and right midbrain) as well as an additional five regions (right posterior inferior frontal gyrus, left orbital gyrus, bilateral posterior lingual gyri, and the right posterior medial temporal fusiform gyrus) (Indefrey & Levelt, 2004).

While it is acknowledged that the production of fluent sensible speech requires linguistic knowledge and inputs from additional cortical regions, Broca's area still has a central role in the production of speech, and through incorporating other regions of the brain (specifically motor cortices), the human brain is able to organise an array of intricate muscle groups in order to transform the lexical representation of a word into a fluent utterance.

Wernicke's Area

Wernicke's area is the cortical region traditionally believed to be responsible for the majority of language comprehension tasks (Rosenzweig et al., 2002).Like Broca's area, Wernicke’s area was named following the discovery that damage to this distinct cortical region can cause to language comprehension. Carle Wernicke, a German neurologist and psychiatrist discovered that damage to the posterior third of the left STG (now known as Wernicke's area), led to the development of a receptive language disorder (Wernicke’s aphasia). During aural language processing, Wernicke’s area is understood to be the first location in which speech sounds are determined to be meaningful words. Not just limited to processing speech streams, Wernicke's area is also responsible for primary processing of the lexical and semantic components of both visually and aurally perceived language. Linguistic information processed in Wernicke’s area is then projected to a number of

specialised cortical regions. The majority of language information is projected across the Sylvian fissure (the prominent divide along the superior section of the temporal lobe) and through the arcuate fasciculus. Linguistic information may also be projected to the cortical regions surrounding these primary areas (i.e., Wernicke's area and the STG) to higher-order association areas in the parietal lobes (Rosenzweig et al., 2002).

Authors such as Boatman (2004) have further investigated the role of cortical regions involved in language comprehension, and suggest that attributing the many intricacies of language comprehension and perception to such generalised areas of the brain is insufficient. Boatman outlines a number of specific areas of the STG and the surrounding areas, and their involvement in language perception. Acoustic-phonetic processing, the discrimination of speech sounds based on phonetic features (e.g., differentiating the phonemes in speech sounds /br/ and /ba/), is one of the earliest stages of speech perception and Boatman reports that specific neuronal clusters in the middle posterior region of the STG are activated during this stage of processing. For these sounds into to be joined words, these basic phonetic components undergo more advanced phonological processing. Boatman reports that phonological processing activates the same cortical regions as acoustic-phonetic processing, but also extends into middle anterior, ventral, and dorsal regions of the STG, as well as the inferior parietal lobe, and the inferior section of the frontal lobe. Following phonological processing, lexical-semantic systems are engaged to derive word information and meaning. Again, Boatman suggests that lexical-semantic processing engages areas activated in previous stages, but that activity also extends to the posterior and anterior STG, the middle and inferior temporal gyri, and the inferior frontal and parietal gyri.

Gestural Origins of Language

In the opening sentence of his article on the evolution of language, Hewes (1976) states "one of the most plausible glottogonic models assumes that the initial form of language was gestural, in the sense that the propositional, predicative, or reporting functions were based on gestural signs, with vocal sounds serving much as they do for nonhuman mammals, for the communication of social affect"(p.482). Hewes notes that the 'modern' view of the gestural theory of language aligns with the notion that gesture offers language a "line of least biological resistance, such that the initial appearance and early development did not require new anatomical structures or behaviour patterns previously impossible" (p. 482). Of course, since Hewes authored that paper, the scientific understanding of the cortical networks responsible for language and gesture has greatly advanced (e.g., Holle, Gunter, Rüschemeyer, Hennenlotter, & Iacoboni, 2008; Hubbard, Wilson, Callan, & Dapretto, 2009), allowing the development of more comprehensive theories on the gestural origins of language.

A modern, and widely published proponent of the gestural theory of the evolution of language is Michael Corballis (2002, 2003, 2004, 2009a, 2009b, 2010). Much of Corballis' work focuses on reviewing the evolutionary changes that occurred in the lead-up to the emergence of language and gestural communication. Corballis (2009) indicates that one such evolutionary advance that split modern day chimpanzees from hominids was bipedalism. Through standing upright, bipedal adaptation allowed ancestors of modern humans increased use of their forelimbs and hands for an array of tasks not as easily performed by their forebears (such as improved reaching and carrying ability). Corballis (2002) points out that he does not believe that manual communication was the adaptive advantage that led to advent of

bipedalism, rather that bipedalism created the opportunity for the hands to be used in the production of gestural communication. However, Corballis also notes that manual gestures would not have developed into a true syntactic language until approximately four million years later with the emergence of the genus *Homo* (Corballis, 2002).

When examining theoretical accounts of the evolution of language, it's important to be aware of the lack of direct empirical evidence. Corballis (2002) himself admits that whether language is "vocal or gestural, (it) leaves little trace in the archaeological record" (p.83). While this implies that many of the theoretical underpinnings are somewhat conjectural, to support the gestural theory on the evolution of language Corballis looks to corroborating evidence from modern day primates and the fossil record which imply adaptive changes in brain and body architecture that allowed for the evolution of language. The evolutionary trajectory from early primates to *Homo sapiens* contains a number of important changes which set the neural foundations for language development. These include increases in brain volume (while maintaining a proportionately smaller body size compared to Neanderthals), and reduction in gestation length (leading to increased infant brain development while exposed to the environment) (Corballis, 2002).

Although this may be considered evidence that the brain had evolved to a stage where it was possible for language to emerge, it does not specifically point to the initial emergence of gestural or verbal communication. Corballis (2003) proposed that the development of language itself may have started well before speech was the medium used to communicate it. Based on numerous examples from the fossil evidence, Corballis states that until the physical changes began to evolve in the human tongue and thorax (approximately 2 million years ago with the emergence

of *Homo habilis*) articulate speech would certainly have been impossible. Additionally, although the evolution of vocal apparatus in the mouth and throat allowed for more refinement of vocal calls, the physiological changes needed for *articulate* speech occurred more recently in human evolution (Lieberman, 1984), as did changes in cortical organisation required to support intentional control of vocalisation (particularly the development of Broca's area) (Corballis, 2002).

Language is arguably one of the most defining features of being human. The ability to communicate information and ideas between individuals provides an great evolutionary advantage, as it allows specific communication about information regarding predatory threats, resource location and availability, as well as the conveyance of information across intergenerational boundaries. Due to the selective evolutionary advantage presented by language and its complex syntactical structure, Corballis (2002) reasons that the roots of language likely predate the emergence of physical structures required for refined verbal articulation. Instead, Corballis believes that the emergence of language over the past 2 million years would have largely taken the form of manual hand movements and that human ancestors would have used gestures to communicate rather than speech. In addition to the fact that other modern-day primates are better equipped to make fine voluntary hand movements than intentional vocal sounds (Corballis, 2002; Enard et al., 2002), Corballis believes that gestures provide a distinct advantage to hunting parties. Using gestures while hunting allows a more silent approach to prey and is logical considering much of the information communicated in hunting (such as direction and location) lends itself to gestural transmission. The !Kung San people of Southern Africa are an example of present day hunter-gatherers who use animal calls to

coordinate stalking prey, however they revert to silent gesturing when within visual range to close-in on the prey (Lee,1979 as cited in Corballis, 2002).

But why the switch to speech? Burling (2007) points out that lack of explanation for a switch from gestural communication to speech is "one nearly fatal flaw" for the gestural origins of language (p.123). Burling asserts that the circumstances required for the transition are unaccounted for, and that "once a visible language was established...a new and initially clumsy vocal language would have offered poor competition for an established system of gestures" (p.123). Corballis (2009b) argues that the move from manual gestures to speech is not actually that great a distance if one considers speech as initiating from a series of orofacial gestures (articulated by co-ordinated movement of the lips, velum, larynx, and tongue) accompanied by an acoustic signal to assist with disambiguation of the message. Corballis therefore surmises that “if this theory is correct, the perception of speech might therefore be considered a natural function of the mirror system” (2009b, p.24). Speech also offers many other advantages not available when gesturing. As bipedalism freed the hands for more adaptive reaching and carrying tasks (as well as gesture), speech frees the hands from communication so they may be used for concurrent tasks, such as the use of tools (an evolutionary trait which is thought to have emerged approximately 2.6 million years ago [Semaw et al., 2003]). Gestures are also entirely constrained by visual limitations placed on the individuals communicating, in other words, gestures becomes an ineffective communication medium when the individual is obscured, far away, or most importantly, when it is dark.

Genetic research is also valuable in understanding the evolutionary emergence of speech, particularly the role of the *FOXP2* gene. The *FOXP2* gene has

been implicated as being involved in speech ability as there is evidence that a mutation of this gene in a family in the UK has impacted their vocal articulation (Watkins, Dronkers & Vargha-Khadem, 2002) and Broca's area function during covert verb production (Liégeois et al., 2003). Beyond humans, *FOXP2* has been found to play a role in song imitation in songbirds (Haesler et al., 2007 as cited in Corballis, 2009b) and synaptic plasticity and motor learning in mice (Grozner et al. 2008 as cited in Corballis, 2009b). Emergence of human lineage-specific changes in the *FOXP2* gene are estimated to have occurred approximately 200,000 years ago (Enard et al., 2002), which Corballis argues is within reasonable temporal proximity to the emergence of *Homo sapiens* approximately 170,000 years ago.

Christiansen and Kirby (2003) review a number of conditions crucial to the evolution of language, that do not necessarily align specifically with speech or gestural theories. Christiansen and Kirby suggest that for language to exist in its current form, humans must have had to possess fundamental attributes central to effective communication aside from the cultural need for communication. Specifically, these attributes may have included the ability to use symbols and attach arbitrary speech or gesture 'symbols' to a vast and ever-expanding array of specific entities; the ability to use joint-attention (follow eye-gaze) to ensure communication focuses on a mutually understood target; the ability to imitate language actions (whether they be speech sounds or hand movements); as well as cognitive enhancements such as sequential and hierarchical learning, the ability to use syntax and grammar, and the understanding of complex and abstract concepts. Christiansen and Kirby point out that some of these skills are available to other primates, however the qualitative and quantitative measure of their utilisation in communication is unique to humans.

As demonstrated above, there are a number of evolutionary and anthropological changes that needed to have occurred in order for language to have evolved in human ancestors, and while Christiansen and Kirby (2003) provide an overview of the cognitive changes that must have occurred over the course of millions of years of evolution, other authors such as Arbib (2002) provide more detailed hypotheses of the neural changes that must have occurred in order to give rise to language. Arbib theorises that the evolutionary roots of language lie in the mimicry of action, particularly the mimicry of hand movements such as grasping and pointing. He suggests that rather than the human brain evolving 'pre-equipped with language', pressures of natural selection directed the evolution of the brain in such a way that it became 'language-ready'. Thus, the brain evolved mechanisms that offered the capacity to use language, so that early humans could advance their communication when the social/cultural need arose.

The framework for Arbib's (2002) theory on the gestural origins of language is thoroughly integrated with research into the mirror neuron system. As reviewed previously in this thesis, the mirror neuron system is a proposed network of neurons that are believed to be responsible for the human ability to draw meaning from the actions of others (see Rizzolatti & Craighero, 2004, for a review). Akin to the evidence cited by Corballis (2002), in light of the inability to investigate the linguistic skills of our forebears Arbib's theory is informed by research on modern-day human mirror neuron systems and extrapolated data from primates that share a common lineage. In this way, Arbib is able to speculate on the abilities of common ancestors of both humans and monkeys who possessed cortical structures that laid the framework for the development of language.

Arbib (2002) hypothesises seven steps for the evolution of language.

1. Grasping,
2. A mirror system for grasping,
3. A simple imitation system for grasping,
4. A complex imitation system for grasping,
5. A manual based communication system,
6. Speech (production and perception of vocal gestures), and
7. Language.

According to Arbib, the first critical stage to the development of language was the evolution of the cortical areas responsible for grasping behaviour. Specifically, Arbib identifies the F5 and anterior intra-parietal sulcus as being critical parts of a cortical circuit that utilised the visual properties of an object and allowed early primates to calculate appropriate grasping behaviour. The subsequent transference of motor information from F5 to the primary motor cortex and other cortical areas, then allows accurate execution of grasping movement based on continuing visual/motor information. In modern day monkeys, F5 neurons can be classified into different categories based on the neuronal activity seen when the monkey carries out different actions (see Arbib, 2002, for a review). Interestingly, these neurons are activated by a broad action directed toward a goal and not activated by the individual motor movements that make up each complete action. Most commonly, these groups can be approximated into: 'grasping-with-the-hand-and-mouth' neurons, 'grasping-with-the-hand' neurons, 'holding' neurons, 'manipulating' neurons, and 'tearing' neurons (Rizzolatti et al., 1988). Arbib is careful to point out that these classifications do not equate to a 'vocabulary of motor schemas', and that while the F5's role in grasping behaviour is central to his theory

of language evolution, the successful execution of grasping movements is a complex process involving other central nervous system components, additional cortical areas (including the basal ganglia, anterior intra-parietal sulcus, and supplementary motor area), and the limbs.

In stage two, Arbib (2002) proposes the development of mirror neurons within the F5 (or an ancestral homologue thereof) provided an observation/action matching system for grasping movements. In monkeys, F5 mirror neurons have been shown to be activated by object-directed action, that is, actions which involve a biological effector (such as a hand) interacting (or moving to interact) with an object (Gallese et al., 1996). Importantly, mirror neurons are only activated when the observed action is object-directed, as observing non-object directed hand-movements, or observing an object independent of action, will not evoke a response from mirror neurons. For example, to activate mirror neurons, a monkey has to see a hand grasping or moving with the intent to grasp an object. Furthermore, not all F5 neurons can be classed as mirror neurons, the difference being that while other F5 neurons may fire when executing action, only mirror neurons will also fire when observing action (Arbib, 2002). Extending the research to the human brain leads to the conclusion that unlike monkeys, the human equivalent of mirror neurons can also be activated by actions that carry no inherent meaning. The human homologue for the monkey F5 is approximated to be Broca's area (in the left hemisphere) (Rizzolatti & Arbib, 1998). PET studies have revealed that when human participants observed grasping movements, significant activation was seen in Broca's area, as well as in other left hemisphere cortical regions including the superior temporal sulcus and the inferior parietal lobe (Grafton et al., 1996; Rizzolatti et al., 1996). Arbib argues that if the similarities between the monkey F5 and the human Broca's area are accurate, then it

is reasonable to propose that a similar cortical area also existed in a common ancestor.

Stages three and four include the development of the neural architecture that allowed human ancestors the ability to imitate both simple and complex action sequences. Arbib (2002) argues that stages three and four bridge the gap between a monkey-based mirror neuron system responsible for matching of action observation/execution information and the evolution of a 'language ready' brain. For this to occur, the mirror neuron system needed to evolve beyond observation of simple grasping actions, and needed to adapt to allow the mimicry of compound action sequences. Arbib suggests that while a mirror neuron system adapted to cope with *simple* action sequences was likely present in ancestors common to both monkeys and humans, the evolution of a mirror neuron system capable of processing *complex* action sequences likely occurred at a point in primate evolution where human and monkey lineages diverged. Arbib also highlights that this adaptation was a 'key innovation' in the evolution toward language. Despite the difficulties in defining the boundary between simple and complex actions, Arbib argues that chimpanzees (and presumably the common ancestor of chimp and human) have the ability to execute 'simple imitation', or the ability to imitate short, novel sequences following repeated exposure. Complex imitation, on the other hand, is the ability to imitate longer novel sequences following a single trial. To provide evidence for the notion that complex imitation is a trait unique to human lineage Arbib cites research that examines the ways chimpanzees learn to use tools. Boesch(1991) observed wild chimpanzees and the way they acquire the ability to crack nuts with stone tools. Mothers were observed to correct infant monkeys as they learned to use stones as tools to crack nuts. This skill is not mastered until adulthood, and Arbib believes that

the extensive observation, correction, and trial and error, needed to master this vital skill is evidence of the long and laborious way they learn to use tools through imitation. By contrast, humans are able to learn more complex motor sequences with much less effort. Arbib states that humans can link individual simple functional units within a sequence in order to successfully execute a more complex sequence. Through practice, previously 'complex' motor sequences become simpler to execute, allowing the incorporation of novel actions that extend the motor sequence (Arbib, 2002). Following the evolution of a system that allowed the sequencing of complex actions, Arbib argues the brain could finally be considered 'language-ready', and although no actual 'language' had emerged, the neural networks (including the mirror neuron system) were now in place that allowed human ancestors to view, understand and replicate hand actions.

Following the evolution of a mirror neuron system that allows learning of complex action sequences, Arbib (2002) proposes the fifth stage of language evolution is the utilisation of this system in the establishment of a manual-based communication. Arbib outlines five hypothetical sub-stages that may have occurred in the development of manual-language.

1. Practical goal-directed actions toward an object.
2. Imitation of those actions.
3. Miming those actions in the absence of the goal object.
4. Development of abstract gestures based on earlier object-directed actions. Arbib suggests that this would occur when the communicative capacity of pantomiming was exhausted.
5. The use of abstract gestures to form compound gestures that can be assigned meanings.

Arbib (2002) suggests that many of the changes occurring across these five sub-stages would likely have occurred in pre-human hominids, however with the emergence of *Homo sapiens*, the advancement that would have occurred in the last sub-stage would have been the most substantial in crafting communication into a form ancestral to what is known as 'language' today. Arbib also acknowledges that when the *Homo sapiens* species had been established he does not assume these individuals had full linguistic capabilities, but that after this stage subtle neural evolution unobservable in the fossil record would have continued to occur. Therefore, further evolution of language occurred not because of substantial changes in neural architecture, but due to what Arbib terms the likely 'cultural evolution' of human beings.

Arbib's (2002) sixth stage marks the transition from manual gesture to speech. As noted above, there are some distinct advantages to adding speech to communication rather than conveying a message by gesture alone (Corballis, 2002), and Arbib outlines the possible evolutionary changes that needed to occur to reduce the prominence of gesture and allow speech to become the primary communication method. As the region of the monkey brain responsible for their calls, while still in the left hemisphere, is distinct from F5 (homologous to Broca's area), Arbib hypothesises that a distinct manuo-brachial (hand-arm) communication system would have evolved in early humans (who would have still used a limited set of species-specific vocal calls). This manuo-brachial system would be established as complementary to systems responsible for primate calls and orofacial gesturing, so that both of these communication systems would run concurrently. Following this, Arbib proposes that area of the brain (assumed to be modern day Broca's area) was established to mediate information via the orofacial and manuo-brachial

communication systems. Finally this system, which incorporates manual-orofacial communication, recruits vocalisation. As to why Broca's (or an earlier iteration thereof) was used for speech and not the 'primate-call' cortical area, Arbib suggests that this is because the manuo-brachial system had already established a system of language in the form of abstract representative manual symbols (gestures). The 'lower-level' task of moving lips, larynx, and diaphragm, etc. to produce vocalisation could be managed by the motor-cortex as the need for primate calls was replaced by speech. Again it is important to note that Arbib does not imply that early man was endowed with fully expressive human language, but simply that the brain and body were at an evolutionary stage that allowed for an extensive cultural evolution giving rise to rich intricacies of modern human language.

The seventh step in Arbib's (2002) theory of the evolution of language is the final emergence of language itself. Arbib suggests that the basic component of modern human language is the verb-argument structure, which allows the creation of sentences such as “Hank pursues Walt" with ‘pursues’ as the verb and ‘Hank’ and ‘Walt’ as the arguments. To reach the verb-argument level of language Arbib outlines the following four steps:

1. Acting on objects (e.g., grasping a flower)
2. Recognising that specific actions relate to specific objects (e.g., recognising that despite similarities, grasping a flower requires a different action compared to grasping a pebble)
3. Creating unique symbols linked to specific action-object relationships. For example, while grasping a flower and grasping a pebble may look similar, they are different and therefore require distinct symbols to differentiate them in discourse (e.g., different

orofacial, hand, and possibly vocal gestures to illustrate different action-object relationships)

4. Naming and classifying actions and objects. This involves creating vocal or manual symbols to establish classes of 'verb-like words' or 'noun-like words' that can be used independent of a specific action-object relationship (e.g., the symbol for grasping a pebble could be extended and applied to grasping a peanut if discourse allowed recognition of a single symbol to represent grasping small rounded objects).

Arbib (2002) suggests that the ability to compound many prototypical verbs and nouns gave rise to the establishment of the verb-argument component of language, and through anthropological and cultural changes there was an increase in the number of verbs and nouns as well as increases in linguistic complexity and diversity with the inclusions of adjectives and conjecture.

Arbib's (2002) theory of the evolution of language provides a comprehensive hypothesis of the evolutionary cognitive changes that may have taken place for non-linguistic human-monkey ancestors to make the transition to the language-capable early *Homo sapiens*, which in turn gave rise to fully linguistically-able modern *Homo sapiens*.

Speech: The Alternative Approach

An exploration of the origins of language would not be complete without a brief overview of the theories that indicate vocalisation and speech were the starting point for human language. Considering this argument broadly, the fundamental underpinnings of the speech theory of language evolution are that early primates,

who already had an expansive array of vocal noises, moved to attribute their calls to specific entities. For example, modern day chimpanzees have an array of vocal hoots, barks and grunts to communicate that food has been discovered, and these vocalisations vary based on the audience (Fedurek & Slocombe, 2013), but at what evolutionary point these somewhat specialised vocal calls became language is debatable.

Hauser et al.(2002) draw comparisons between the highly refined language of humans, and the communication that exists in many organisms throughout the animal kingdom (such as primates, whales, and birds). Hauser et al. point out that while there is a clear distinction between the broadly limited, repetitive nature of animal communication and the human ability to have access to fully expressive, creative and open language, there are many non-human organisms that have access to communication skills that overlap with those of humans. An example is the observation that some bees can communicate displacement via their 'waggle-dance' which indicates the location of a distant food source (Riley, Greggers, Smith, & Reynolds, 2005). Hauser et al. propose that many of these 'peripheral systems' such as adaptations to the sensory-motor systems (those that allow speech or sign) and conceptual-intentional (problem solving) systems have evolutionary roots pre-dating the emergence of language, and while these systems are necessary for language to exist, the development of true human language did not occur until our early ancestors developed the ability to use *recursion*. According to Hauser et al., recursion (the ability to place one phrase within another phrase of the same type) is a uniquely human trait that allows the infinite generation of novel utterances – a trait that truly makes language the domain of human beings. As to what selective environmental pressures led to the evolution of this defining trait of human language, Hauser et al.

suggest this is a question that remains to be answered. Other researchers, such as Everett (2007) have suggested that not all human language contains recursion (such as that of the Amazonian Pirahã people), and therefore argued that recursion may not be the sole trait that sets human language apart from animal language.

As to how human ancestors moved from simplistic vocalisation to language, evolutionary "game theory" modelling such as that conducted by Nowak and Krakauer (1999) may provide some insight. Not unlike genetic competition for survival, Nowak and Krakauer propose that vocal sounds 'compete to survive'. That is to say that to 'win' the competition against other words, specific vocal noises would be used more regularly by multiple parties in order to be attributable to specific entities or classes. Survival of vocal sounds depends entirely on their continued use, and as long as a vocal sound continues to be used and it communicates something to the listener, then it survives. Nowak and Krakauer point out that there is obviously a limit to the number of vocal noises an individual can make, which places quite heavy limitations on the survival of speech. However, by combining multiple sounds into unique sequences, early humans were able to overcome this limitation and create seemingly endless connections between sound-sequences (or 'words'), and their relationship to objects, actions, etc. The authors point out that for language to have emerged, it was necessary that there would be some survival advantage to the individuals using it. For example, a group of individuals who have a vocal sound-sequence alert that distinguishes a snake from a lion would be better prepared to deal with such a threat than a group that merely had a generic vocal call for danger. It is important to note that the authors are not suggesting that speech and word usage emerged within a generation, but that it evolved over a long stretch of time like any other trait. Nowak and Krakauer indicate

that the use of language to enhance survival is the likely impetus for the development of grammar and syntax. Their reasoning is that the ability to put words in a consistent and comprehensible order conveys extra information beyond simple one word utterances (e.g., understanding the difference between "man hunts leopard" and "leopard hunts man" can dramatically change the behavioural response). It is important to note that, despite the focus on prioritised evolution of speech, many of the steps suggested in this theory may also be applicable to the evolution of gestural communication. For example, the notion of 'successful' sounds or words could also be applied to useful informative gestures, as these too would also potentially increase the survival chances of a community of communicating individuals. These reservations aside, from a computational point of view Nowak and Krakauer's game-theory of the evolution of language is a plausible alternative to the gestural theory of language evolution.

If theories of the gestural origins of language are accurate, then it is also important to identify what modern day artefacts of the once-prominent gestural communication system may still exist in modern humans. An example of a modern indicator of language's gestural origins lies in the phenomenon of right-handedness and the relationship between handedness and left-hemisphere language dominance. The majority of people are born right-hand dominant (Annett, 2002), meaning that the right hand is preferentially used to complete many skilled motor actions (such as writing or throwing). The contralateral motor cortex is largely responsible for controlling the actions of the right hand and as mentioned above, and the vast majority of language processes are also ascribed to regions of the left hemisphere (such as Broca's and Wernicke's areas). Researcher investigating the links between language and handedness have found that language is left-hemisphere dominant in

90-95% of typical right-handed people (Knecht et al., 2000). However, according to Knecht et al. the relationship between handedness and language dominance is not symmetrical and despite left-handers being in the minority, 71-77% of left-handers are also left hemisphere dominant for language. Further research conducted by Pujol, Deus, Losilla and Capdevila (1999) revealed that even in right-handers, not all language processing occurs in the left hemisphere and bilateral activation is seen in approximately 4% of individuals. Corballis (2002) reviews many aspects of lateralised specialisation in an array of species beyond human beings. He cites many examples of left-hemisphere dominance for vocalisation in other primates, birds, and even frogs (who, as Corballis notes, are not even endowed with a cerebral cortex). Like Arbib (2002), Corballis is very careful to specify that vocalisation is *not* language, however in accordance with the gestural theory of language evolution, the notion that vocalisation, dexterous refined manual hand movement and language should all be in the same hemisphere is unsurprising. As for the existence of asymmetry, Corballis attributes this to the finding that asymmetry exists in some of our most distant ancestors (e.g., birds and frogs), and broadly links this to evolutionary selection pressures. These selection pressures have afforded many symmetrical features where necessary or when it improves survival (limbs, wings, eyes, kidneys, somatosensory cortices, etc.), and limited the space allocated to features that would not necessarily improve survival through lateralisation (e.g., vocalisation or limb dominance).

Other artefacts that may indicate a gestural origin for language include the current use of gesture in modern communication, and the notion that gesture and language are processed together. Arbib's (2002) theory lays the groundwork for the notion that gesture and speech are not two distinct systems, but are in fact part of a

unitary system of communication that includes three parts: hand gesture, speech, and orofacial movements/expressions. This singular system model allows for the integration of a number of observations. These include the fact that the majority of childhood language acquisition occurs in the domain of speech, that deaf individuals can utilise a fully functional manual language, and that gestures exist and have a close relationship to speech. An exploration of the linguistic nature of gestures and their connection with speech is explored in more detail in the next chapter.

Chapter 4: Gesture

In many cases, speaking is often accompanied by movement of the hands and arms, known as gesture. This chapter aims to provide a brief overview on the theoretical and empirical literature on gesture, with inclusion of research that covers the behavioural aspects of gesture production, as well as studies that examine the underlying neural correlates such as neuroimaging and EEG studies. McNeill (1985) defines gestures as "movements that occur only during speech, are synchronised with linguistic units, are parallel in semantic and pragmatic function to the synchronised linguistic units, perform text functions like speech, dissolve like speech in aphasia, and develop together with speech in children' (p. 351).

Rossini (2012) identified five common types of gesture used to accompany speech: emblems, beats, deictic gestures, iconic gestures, and metaphoric gestures. Emblems are gestures that are lexically arbitrary and are based on cultural predetermination rather than associated meaning. An example of an emblem is the 'okay' hand gesture (making a thumb-to-forefinger circle with the remaining fingers extended), but as emblems can be interpreted in the absence of speech, McNeill (1985) does not consider them to be a true gestures. Beat gestures refer to rhythmical hand movements that usually hold no inherent meaning, but occur sporadically alongside the prosody of speech. An example of a beat gesture would be repeatedly tapping a fist in mid-air (as if shaking dice) while expressing that you are trying to recall something from memory. Deictic gestures usually refer to pointing gestures used to indicate objects or people within the conversational space. An example of a deictic gesture would be to point to a space earlier allocated to a character in the spoken narrative while saying "...and then *she* found twenty dollars". Iconic and

metaphoric gestures are similar in that they both convey meaning about the accompanying speech stream. Iconic gestures usually link directly with the meaning presented and are not culturally limited, such as patting the abdomen while saying "I'm really full". Metaphoric gestures, on the other hand, often refer to more abstract concepts associated with the speech stream, such as saying "I think I've heard enough" while holding up a flat palm to the listener indicating them to stop. Rossini indicates that there is some controversy surrounding the distinction of iconic and metaphoric gesture, and based on McNeill (2007), she suggests that the descriptors may be interpreted more broadly as dimensions rather than discrete categorical entities. For the purposes of this thesis, the term *iconic gestures* will encompass both the iconic and metaphorical definitions given above.

Gesture, Speech, and Cognition

Due to the semantic relationship between iconic gestures and speech, the sorts of gestures introduced above are often referred to as co-speech gestures (Holle & Gunter, 2007; Kelly, Creigh, & Bartolotti, 2010; Wu & Coulson, 2005). Unlike speech, which is conventionalised and largely arbitrary (with a few exceptions such as onomatopoeia), gesture is idiosyncratic and imagistic (Kelly et al., 2010), but it is also highly reliant on speech to provide context and to allow its meaning to be understood. Iconic gestures are also likely to provide additional semantic information to discourse, encouraging the listener to develop an enriched conceptual representation of the speaker's message (Wu & Coulson, 2005). McNeill (2007) theorises that the source of meaning for gestures is the imagery they create. An example of this could be a brisk palm-down open handed sweeping gesture when saying "I'll get to that later". In this example, the gesture does not change the essential meaning of the speech stream, but it does convey that the speaker is

metaphorically 'clearing away' the information/task they have just been given to focus on the task at hand, perhaps even with the implication that the new task is not a priority for them. Kelly et al. (2010) offer another example: gesturing a drinking motion while saying "They were up late last night". In this example gesture adds the information that they were up late last night because they were drinking alcohol. In some cases gesturing in this way might also give the added implication that the additional information is not something the speaker is prepared/allowed to speak about.

McNeill (1985) argues that gesture and speech are "parts of the same psychological structure and share a computational stage" (p. 350). McNeill notes that an earlier view was that anything *linguistic* could be written down, whilst everything else is considered non-linguistic. According to this view, this division is an arbitrary cultural artefact and gesture is actually cognitively connected to speech. McNeill (2005) describes gestures as co-expressive, but not redundant, in that gesture and speech both convey the same underlying concept, but express it in their own way. Importantly, McNeill (2007) also argues that the synchronicity of gesture and speech at the time of co-expression is crucial as at the moment of speaking, it indicates that the mind is approaching the same underlying concept in two different ways.

A fundamental part of McNeill's (1985, 1992, 2007) thesis is that co-speech iconic gestures, like speech, carry meaning that adds to a listener's understanding. McNeill (1985) illustrates the use of co-speech gestures in an example in which he recorded gestures made by five adult participants as they recalled a scene from a cartoon in which a character moves up a drain-pipe. Although these responses were not quantifiable, McNeill (1985) believes that the commonalities he observed in gestural patterns across participants, such as all participants including a gesture with upward

movement (to demonstrate the character's motion) indicate that gestures are fundamentally linked with processing linguistic concepts in the gesture producer. Further, McNeill (1985) found that even when participants used different words (such as *drainpipe*, *drainspout*, *rain barrel*, or *rain-gutter*) their gestures (in this case an upward facing hand flexion/hand-basket shape) were still comparable. McNeill (1985) states that gestures share characteristics with speech. For example, gestures are similar between individual speakers when the meanings conveyed are similar, and when the meaning changes, the hands produce extra small movements to mark the boundary between one meaning and the next. McNeill (1985) acknowledges that gestures does not have the same complexity as speech, and that complex meanings are divided between the speech and gesture channels and that ultimately speech and gesture cooperate to present a simple complex meaning.

Kelly, Kravitz, and Hopkins (2004) indicate that there is debate in the literature regarding the integrative nature of speech and gesture, and while it is generally acknowledged that gesture plays an important role during language production, there is a question over whether gesture production is solely beneficial to speaker (via mechanisms such as enriching encoding of information, message conceptualisation), or whether gesture also serves a communicative function for the listener. Holle and Gunter (2007) conducted a study examining the utilisation of gestures when disambiguating speech streams. These researchers presented participants with a phrase with ambiguous target words such as "ball" which was accompanied by a gesture indicating playing a ball game (the dominant interpretation of the target word) or a gesture indicating dancing (the subordinate interpretation). Using EEG evidence (which is covered in more detail later in this chapter, p. 62), Holle and Gunter found that the presence of the gesture assisted with

the disambiguation of the target verb, supporting that notion that gestures can aid in the comprehension of language. Goldin-Meadow (1999) points out that this type of communicative act is likely to be the most useful function role of gesture to the listener; that is, gestures' value can be found in the role they play to disambiguate the speech stream, where the information presented in speech does not convey the full intended meaning of the speaker.

Not all researchers agree with the assistive role of gestures in language comprehension, however. Krauss, Dushay, Chen, and Rauscher (1995) designed a study to examine the communicative value of hand gestures when used in conversations. The study consisted of encoding and decoding phase. In the encoding phase, participants were videorecorded while describing an abstract graphic design, or a novel sound to a confederate partner (the "listener"), and the listener was required to choose the correct target from an array of choices. In half the trials the participant and the listener sat face-to-face during the target description, whereas in the remainder of the trials, descriptions were conducted via an intercom. Regardless of the communication type, the description was still videorecorded. Krauss et al. found that rate of gesturing was higher for descriptions of the graphic designs compared to the sounds, and also higher for face-to-face communication compared to the intercom. In the decoding phase, a different set of participants were tasked with determining the target design/sound being described in the original recordings. Half the participants watched the video recordings (which offered both audio and video), which the remainder listened to audio recordings only. They found no significant differences between the accuracy in the two conditions regardless of whether participants were describing abstract graphic or novel sounds. Specifically, participants identified the correct target 65% of the time when presented with audio

descriptions only, and were correct 66% to 68% when given combined video-audio descriptions. As participants were equally accurate regardless of whether gestural information was present, Krauss et al. interpret these results as evidence that gesture does not add any beneficial communicative information to the listener.

In an earlier study Krauss, Morrel-Samuels, and Colasante (1991) demonstrated the necessity for speech to be present during the presentation of iconic gestures. In this series of experiments, the researchers asked participants to view and identify gestures in the absence of speech in a range of conditions (such as with and without context). Krauss et al. found that while overall performance in gesture recognition was better than chance, accuracy was substantially better when speech was presented in isolation, or at the same time as gesture. Within the framework of McNeill's (1985) view these results could be indicative of the underlying the notion that gestures and speech occur together as a communicative act, however Krauss et al. concluded that speech is the most important part of communication and that gesture is largely redundant to communicating a message if intelligible speech is present. In more recent research, Hadar and Pinchas-Zamir (2004) investigated the notion of the "semantic specificity" of gestures; that is, the degree to which a particular gesture indicates the meaning associated with it. In that experiment, participants viewed video clips of a person uttering a phrase and producing a gesture. The videos were presented in three different ways; video information only (no audio), video plus on-screen text (subtitled speech stream with target word omitted), and video plus on-screen text and speech (same as previous condition with the addition of an audio speech stream with target word omitted). Additionally, the gestures demonstrated in the clips were coded by the researchers as either being iconic (i.e., gestures that show in their form that they are related to their meaning),

conventional (i.e., gestures that carried a fairly clear meaning, but contained some arbitrariness, and indefinite (i.e., gestures that resembled iconic gestures, but were not related to the speech stream). Participants had to choose the meaning from a list of five possible answers including the correct answer as well as some semantically and visually linked distractors. The iconic gestures, perhaps unsurprisingly, elicited the most correct responses, as they were accurately identified 40% of the time, compared to conventional (33%) and indefinite gestures (25%). Interestingly, in the condition in which participants were given the most information (video plus text and speech) with the best formed gestures (iconic) they were only able to accurately identify the gestures 45% of the time. The authors cite this as evidence that without accompanying speech, the underlying meaning of a gesture can be vague and tentative, and that interpretation of gestures is not very specific. Once again, this could be interpreted as supportive evidence for the argument that gestures may not add anything to speech and that they are inherently ambiguous to the listener (akin to the argument presented by Krauss et al., 1991).

If the theory that gesture offers little benefit to the listener's comprehension is accurate, then why do we gesture when we speak? One possibility is that gesture may reflect or even facilitate the thinking that underlies speaking (Iverson & Goldin-Meadow, 1998). In 1996, Rauscher, Krauss, and Chen asked participants to describe a series of high-action cartoon excerpts they had just viewed. Participants were either allowed to freely gesture, or were instructed to keep the hands placed against dummy electrodes under the guise that they needed to hold their hands still for recording purposes. Furthermore, lexical access difficulty was also manipulated by allocating participants to a normal-speech condition (free choice of vocabulary), obscure-speech condition (aim to use as many obscure words as possible), and a constrained-

speech condition (avoid using words that contained a specified letter). Rauscher et al. recorded and analysed participants' responses for a number of characteristics including diversity, spatial content (words/gestures related to movement/space), fluency, and pauses. Overall the authors found that participants were most likely to gesture when their speech was spatially related than when it was not, and that they had more difficulty producing spatially related speech (e.g., poorer fluency and more hesitations) when their freedom to gesture was restricted. Rauscher et al. suggest that this indicates that gestures may facilitate lexical retrieval, particularly when the information is spatially related, or alternatively that gesture suppression affects a speaker's ability to conceptualise spatial relations making it more difficult to verbalise.

In their 2001 paper, Goldin-Meadow, Nusbaum, Kelly, and Wagner take the notion of gesture assisting cognition further, and set out to test the possibility that gesturing during speech saves cognitive resources, making it easier for the speaker to concurrently perform other cognitive tasks. In their study, child and adult participants were required to solve a mathematical problem (simple addition for children and an algebraic equation for adults) on a blackboard. Upon solving the problem, participants were shown a list of items to remember (words for children and letter pairs for adults). After viewing the items, participants were asked to explain how they solved the equation, and during this time participants were either allowed to gesture freely, or were restricted from gesturing (told to keep their hands on the table). Although the authors had an initial sample of 40 children and 36 adults, this was reduced to 26 children and 32 adult participants who gestured when permitted. Participants were required to keep the list items in memory throughout the explanation and then recall them following their explanation. List difficulty was also

manipulated in the study. Participants were either asked to remember a short list of items or a longer list, with the assumption that the longer list would require more cognitive resources and would be harder to accurately recall. While the researchers did not find any significant effects of gesturing on the shorter list, gesturing improved recall for the more cognitively taxing longer list in both adult and child participant groups.

Further to this, Goldin-Meadow et al. (2001) wanted to check that 'not gesturing' does not in itself provide cognitive load. Nine children and 10 adult participants gestured on some, but not all trials, which allowed the authors to compare recall in trials where participants did not gesture by choice with trials in which they were not permitted to gesture. Again it was found that for the longer list, participants recalled more on trials where they gestured during the intervening explanation than when they did not, regardless of whether gesture restriction was forced, or by choice. Goldin-Meadow et al. conclude that recall was easier in the gesture trials because gesture reduced the cognitive load of the equation explanation, freeing up cognitive resources to be applied to memorising/recalling the list of words/letter-pairs. This raises the important question of how gesture might increase available cognitive resources.

One such possibility is that gesture enriches encoding of information, as gesture can convey the same basic idea as speech, and it does so using a visuospatial rather than vocal modality (as demonstrated by Rauscher et al., 1996). A further possibility for why gestures may increase available cognitive resources is the notion that gesture can help speakers organise information for the act of speaking and thus facilitate conceptualisation of the message. In an earlier study, Alibali, Bassok, Solomon, Syc, and Goldin-Meadow (1999) demonstrated that gestures may help

individuals conceptualise a problem and they can be indicative of an individual's mental representation of problem-solving processes. In this study, participants were asked to read aloud and discuss a strategy to solve a mathematical word problem. Some of these problems required the conceptualisation of continuous change, some discrete change, and some could be conceptualised as either continuous or discrete change. Participants' responses were video recorded and hand gestures subsequently coded into continuous representations (e.g., sweeping, arcing, dragging), discrete representations (e.g., a sequence of three or more taps, points, or beats) or neither. Additionally, the strategy verbally explained was coded as being continuous or discrete, and then compared with co-occurring gesture. In 28% of trials, gestures were congruent with speech across both problem-solving strategies. In 45% of trials, gestures were either non-specific or displayed no clear alignment with either problem solving strategy, and in the remaining 28% participants conveyed a different mental representation in gestures compared to speech (e.g., a discrete gesture with a continuous verbal representation or vice versa). It was found that when gesture and speech were congruent, it very clearly indicated the type of problem-solving approach that the participant would ultimately use, and the authors interpreted this as strong support for the notion that gesture and speech form a single mental representation of a problem. However when gesture and speech mismatched, there was much more variation in the final problem-solving approach, and this final approach did not reliably coincide with speech or gesture. The authors suggest that this indicates there is a possibility that speech and gesture can derive from different mental representations of a problem in some cases. Gesture communicates information beyond that conveyed in speech, otherwise speech would be a reliable indicator of final problem solving solution even in mismatched trials(Alibali et al.,

1999). Ultimately, as speech alone is not a reliable indicator of mental representation, these authors have demonstrated that when gesture and speech operate congruently, gesture assists in the conceptualisation of a problem-solving strategy.

As outlined above, gesture is by no means a simplistic addendum to spoken language, and has the potential to enrich communication of a message. Although the intended meaning of a gesture is highly reliant on the accompanying spoken content (Hadar & Pinchas-Zamir, 2004), it provides valuable support to the speech stream. Whether it be for the benefit of the speaker or the listener, the inclusion of co-speech gestures enhance the message by providing additional information (Holle & Gunter, 2007), assisting the speaker in processing information related to the speech stream (Ali-Bali et al., 1999) and potentially reducing the cognitive resources needed for speech production, allowing the individual to allocate resources to other tasks (Goldin-Meadow et al., 2001).

Childhood Development of Gesture

Researchers often look to the development of linguistic skills over the lifespan to yield greater insight into their purpose and function. To investigate whether utilising co-speech gestures was an innate ability or a learned communicative behaviour, Iverson and Goldin-Meadow (1998) compared the gestures produced by 12 congenitally blind children and 12 sighted children. Participants were videotaped while responding to a series of reasoning tasks that had been previously shown to elicit gesturing in sighted children. As expected, the sighted children gestured as they spoke, and remarkably all 12 blind participants also used gestures as they spoke (approximately one to two gestures per reasoning task). Further, both the blind and sighted participants used comparable gestures (such as a

turning C-shaped hand to indicate the transference of liquid from one vessel to another), and the number of gestures used by the two groups was similar. Iverson and Goldin-Meadow also investigated whether the sightedness of the listener would impact gesture production in blind children. Four blind children performed reasoning tasks with an experimenter whom they were told was also blind. Despite this knowledge, the participants still gestured at a rate comparable with that when they believed the listener was sighted. Iverson and Goldin-Meadow acknowledge that the small number of participants in these groups may have made it difficult to detect differences in the quantity of gestures. However the more important point is that all blind and sighted participants produced similar gestures when speaking. As the congenitally blind children had never seen gesture, Iverson and Goldin-Meadow conclude that gesture does not need to be modelled to exist and that it will occur in the absence of an observer (i.e., even when the listener is "blind").

The ability to gesture does not always develop normally, however, and in some individuals, such as children with developmental co-ordination disorder (DCD), gestural ability can be impaired. DCD is diagnosed in children who for no obvious medical reason fail to acquire adequate motor skills. This disorder impacts on the child's manual dexterity and balance, which can in turn affect a range of daily tasks such as handwriting, dressing, and eating (Zoia, Barnett, Wilson, & Hill, 2006). Zoia, Pelamatti, Cuttini, Casotto, and Scabar (2002) compared the development of emerging gestural abilities in children with and without DCD. They looked at transitive gestures, that is, gestures directed towards objects (such as moving a fist from front to back over your head to indicate "combing your hair"). Without accompanying speech, these gestures are quite different from the iconic co-speech gestures referred to throughout this chapter, but even transitive gestures are in

essence still communicative. Each gesture was elicited from the child through four methods of increasing difficulty: *Imitation*, in which the child was required to imitate the researcher performing the gesture; *Visual plus Tactile*, which involved the use of real objects (such as a comb); *Visual*, in which the child was asked to mime the use of a seen object; and *Verbal* in which the child was asked to gesture in response to a verbal command (e.g. "show me what you do to comb your hair"). Zoia et al. (2002) found that children with DCD performed consistently more poorly than age-matched controls, but that the difference between the two groups lessened with increasing age. Zoia et al. (2002) primarily attribute these findings not only to the children's decreased manual dexterity, but also to their impaired ability to integrate sensory and motor information. However, these authors do not expand on the aetiology of the disorder or the specific relationship with gestural production/observation. Werner, Cermak, and Aziz-Zadeh (2012) hypothesise that the impairments in motor planning, motor learning and imitation observed in DCD may be caused by a malfunctioning mirror neuron system, which is suspected to have a crucial role in successfully interpreting goal-oriented actions, including the processing of gestural information (Holle et al., 2008). Werner et al. note that there is currently not enough evidence to confirm the link between DCD and a malfunctioning mirror neuron system. However there is an emerging body of brain-imaging evidence that lends support to the notion that there may be different levels of cortical activity in areas associated with mirror neuron systems (see Werner et al., 2012, for a review).

Neural Correlates of the Interplay between Speech and Gesture

To gain a better understanding of the extent of the integration of gesture and speech, and to understand the possible facilitative effects of gesture of speech, it is important to look beyond observational and behavioural studies (Krauss et al., 1991;

McNeill, 1985). An examination of the literature on psychophysiological measurement techniques gives greater insight into the neural correlates of the interplay of speech and gesture systems.

There have been a range of EEG studies (such as Kelly et al., 2010) which have examined recordings of brain activity when participants are processing gestures and speech. Many of these studies have examined specific components of event-related potentials (ERP), which are distinct waveform patterns identifiable in EEG data that are time-locked to a specific event (such as hearing a word or viewing a gesture). Of particular interest is an ERP component known as the N400 (named as such because it is a negative amplitude potential peaking at approximately 400ms, and usually occurring between 300 to 600ms post stimulus onset [Kutas & Hillyard, 1980]). The N400 peak was initially observed as being evoked in response to incongruent semantic information (Kutas & Hillyard, 1980). Kutas and Hillyard conducted an ERP study in which participants were required to read sentences one word at a time. The sentences ended with target words that were either semantically congruent and followed an expected semantic trajectory, (e.g., "It was his first day at *work*"), or target words that were semantically incongruent (e.g., "He spread the warm toast with *socks*"). Averaged across many trials, they found that the incongruent word ending reliably elicited a substantial N400 peak. Furthermore, the N400 peak was largest when the words were 'strongly' incongruent, compared to words that moderately incongruent (e.g., "He took a sip from the *waterfall*"). To confirm that it was indeed the semantic properties of the word that evoked the N400, Kutas and Hillyard also investigated the effect of physically altering the target words (e.g., increasing the font size of the target word). They found that words that physically deviated produced a different set of positive peaks (P210, P360, and

P560) which appear unrelated to the semantically driven N400. Kutas and Hillyard conclude that the N400 provides useful information about language comprehension, particularly regarding the timing and interactions of semantic classification processes, implying that the temporal location of the N400 indicates that semantic integration does not occur before that point. While the basic understanding of N400 as a marker of semantic integration is largely unchanged, in a more recent review article, Kutas and Federmeier (2011) acknowledge that many researchers have expanded on these original findings, and in turn broadened the complexity of how the N400 is conceptualised.

To extend on the original findings of the classic Kutas and Hillyard N400 experiment (1980), other researchers have investigated changes in N400 in other linguistic tasks such as processing of speech and gesture pairings. Kelly et al. (2004) provide an example of a study that has used ERP component analysis, with a particular focus on the N400, to examine the neural correlates of gesture and speech. In that study, EEG data were collected from 15 participants as they viewed video clips of a presenter describing and gesturing toward objects. In each clip, the presenter spoke one of four words while gesturing toward the objects (a plate and a glass). He either said *tall* or *thin* and gestured to indicate the tallness or thinness of the tall, thin glass, or said *wide* or *flat* and gestured to indicate the wideness or flatness of the wide, flat plate. The speech and gestures were combined differently across four conditions. In the *matching* condition, the speech and gestures were congruent (e.g., said "thin" while gesturing to thinness of the glass). In the *complementary* condition speech and gesture were incongruent, but the gesture indicated the alternative feature of the same object (e.g., said "thin" while gesturing to the tallness of the glass). In the *mismatching* condition, speech and gesture were

again incongruent, and the speech addressed a feature opposite to that applicable to the object being gestured about (e.g., said "thin" while gesturing to the wideness of the plate). Finally, the *no gesture* condition was used as a control, and the presenter did not gesture and only speech was provided. During stimulus presentation, ERPs were recorded in relation to the onset of speech, and participants pressed different response buttons depending on whether the speech referred to the glass or the plate.

Kelly et al. (2004) found a discrepancy in a number of ERP waveform components between the different presentation conditions. Specifically, the N400 peak amplitude demonstrated more negativity in the *mismatching* condition (when gesture and speech were most distinctly incongruent) compared to the *matching* condition. There were no significant differences between *mismatching* and *complementary* or *no gesture* conditions. Kelly et al. report that these results clearly indicate that the presence of gestures influences the way that the brain processes speech, and that this can be used as evidence against the argument that gestures add nothing to communication. It could be suggested that as the N400 is a late component of language processing, gesture affects comprehension only after the brain has already processed some semantic information from the speech stream. However, Kelly et al. also note that there is some discrepancy in ERPs recorded from bilateral frontal cortical areas for the earlier P2 (approx 200 ms) peak, and an even earlier component occurring approximately 36ms post stimulus onset. An analysis of 36 ms peak as well as P2 indicated that there was a larger amplitude for the *mismatching* and *complementary* conditions compared to the *matching* and *no gesture* conditions. Kelly et al. suggest that this is evidence that even at these early stages of processing "gestures appear to influence how speech is acoustically encoded several hundred milliseconds prior to any semantic analysis of speech"(p.

257). As for the difference in waveform profiles between *mismatched* and *complementary* gestures, it appears that only substantially different (*mismatched*) gestures influence later semantic processing. As speech and gestures related to different objects in the *mismatching* condition, they are more incongruent than the *complementary* gestures which merely described a difference feature of the same object and this dissonance between speech and gesture in the *mismatching* drives the larger N400 amplitude (Kelly et al., 2004).

In a more recent study, Kelly et al. (2010) further investigated the cortical activity common to both gestures and speech, and their results speak to the automaticity of gesture and speech integrative processing. In that study, participants viewed one-second video clips of either a female or male presenter performing iconic gestures. Unlike the previous study (i.e., Kelly et al., 2004) in which the gestures were object-oriented (e.g., gesturing tallness of the tall glass), the gestures in the more recent study were truly iconic (e.g., mimicking the opening and closing of a pair of scissors with the index and middle finger while saying "*cut*"). The presenter sat behind a table with the camera framed around the table surface and upper torso, but not the face. Again participants viewed congruent gesture-speech combinations (as the "cut" example demonstrates), as well as incongruent gesture-speech combinations (e.g., presenter performed scissors gesture while saying "*stir*". Additionally, in some trials participants would see a male gesturer and hear a female voice and vice versa. Kelly et al. (2010) indicate that presenting gesture and speech under these sorts of conditions effectively sets up a Stroop-like task which allows for more insight into the automaticity of gestural processing. Testing automaticity in this way allows the researchers to determine whether individuals automatically integrate gestural and speech information when it is presented to them, or whether they

consciously and strategically choose to integrate them. Unlike in the previous experiment (Kelly et al., 2004), participants were not asked to evaluate the semantic content of the speech or gestures, but merely to rapidly identify the gender of the speaker. Asking participants to respond in this manner increase the chances that participants would minimise attention to the semantic content of the gestures or speech, so that any attempts to process conflicting semantic information (reflected in an N400 peak) were due to automatic processes and not attentional biases or effort (Kelly et al., 2010). It was found that there were larger N400 amplitudes and participants were slower in identifying the gender of the speaker when the gesture and speech were incongruent. The larger N400 amplitude suggests that participants were still processing both the gestures and speech, and the delayed reaction time indicates that even though integrating semantic information was not required for the task to be completed effectively, incongruent speech-gesture information caused a response delay. Kelly et al. (2010) conclude that not only are speech and gesture processed together, but that the integration of information from speech and gesture occurs automatically.

Holle and Gunter (2007) also examined the extent to which the brain uses gestural information when processing speech, but in a different manner to that of Kelly et al. (2010). In that study native German-speaking participants viewed videos of a presenter speaking and gesturing simultaneously. Unlike the other ERP studies listed above, the utterances presented were longer and the gestures were executed with speech at a particular point in the utterance, which more closely resembles the way that gestures are experienced in daily life. In the videos, the presenter spoke a series of utterances in German which contained an ambiguous word in the middle, and therefore allowed the sentence to be finished in one of two ways. This

ambiguous word had the potential to be disambiguated via gesture, or alternatively the presenter would gesture in a way that deliberately contrasted the overall meaning of the sentence. The direct translation example provided by Holle and Gunter begins with "*Everybody was impressed by Sandra. She controlled the ball,*" (with ball being the ambiguous word), and concludes with "*which during the game at the serve clearly showed"* or "*which during the dance with the bridegroom clearly showed"*. During the video the presenter would gesture at the point of saying the word *ball* and the gesture would either indicate they were playing with a sports ball, or they would position their arms as if ready to dance with a partner. Similar to other studies, Holle and Gunter had a congruent speech and gesture condition, in which gestures matched the final outcome of the utterance, and an incongruent condition, in which the gesture and subsequent speech were not complementary. Following the conclusion of the video, participants were asked to make a judgement on whether the gesture matched the subsequent speech. EEG data were collected and ERP waveforms time-locked to target words were analysed. In the above example, the target word is "*game*" or "*dance*" and while these words are not occurring during the gesture, they are the first point in the utterance in which the participant is able to determine whether or not the gestured "*ball*" is congruent or incongruent with the subsequent speech. It is important to note that the potential sentence endings were also labelled as dominant (more likely) or subordinate (less likely, but still possible). As expected, akin to the findings of Kelly et al. (2004, 2010), in conditions in which gesture and subsequent speech were incongruent (i.e., dominant gesture with a subordinate sentence ending and vice versa) a larger N400 peak was observed compared to conditions where speech and gesture were congruent.

In an adaptation of the above experiment which was published in the same paper, Holle and Gunter (2007) explored whether or not gestures can actually facilitate a listener's ability to process speech. In other words, do congruent gestures actually make it easier to process speech information, or do incongruent gestures simply make it more difficult? In this experiment, a third condition was added in which the presenter would move their hands in a non-gestural grooming motion (e.g., scratching). The researchers included this as an alternative to gesturing that still requires the participant to attend to a hand movement, but this gesture is non-linguistic and not semantically linked to any specific word in the utterance and therefore does not give the participant clues on how to process the subsequent words. Interestingly, it was observed that when a sentence ended with the subordinate alternative, N400 had a smaller amplitude when following the congruent subordinate gesture compared to both the grooming and dominant gesture. It would be expected that as the grooming hand movement is semantically and linguistically neutral, it would have no impact on the processing of the sentence (and therefore limited impact on N400). Therefore, as the congruent subordinate gesture-speech pairing elicits a smaller N400 than the grooming condition, Holle and Gunter suggest that in this case congruent gesture has reduced the effort required to process the speech stream. Based on the results of both of these studies, Holle and Gunter conclude that gesture assists listeners to disambiguate speech, and in some cases (when the overall conclusion of the sentence is unexpected) gesture can actually facilitate processing.

Beyond EEG, researchers have also used imaging techniques to examine the neural correlates of speech and gesture. In a follow-up to the Holle and Gunter (2007) study, the extended research team of Holle et al. (2008) used fMRI to examine cortical activity while participants were presented with a video that showed

a presenter speaking a sentence with an ambiguous target word (e.g. "*She touched the mouse*") while either producing a meaningless grooming movement or a gesture that supported either the more dominant interpretation of the sentence (touching a small rodent) or the subordinate interpretation (touching a computer mouse). Following the presentation of the gesture sequence, participants had to choose the meaning of the target word from two options displayed on-screen. Throughout the presentation of the video clips, cortical activity was monitored via an fMRI scanner. The researchers found that in the meaningful gestures conditions (both dominant and subordinate) greater cortical activation was seen in the left posterior superior temporal sulcus (STS), and bilaterally in the inferior parietal lobule and the ventral premotor cortex. STS activation has been seen in previous studies where the brain is required to integrate audiovisual information (such as the integration of letters and speech sounds shown by van Atteveldt, Formisano, Goebel, and Blomert [2004]). Although there was some increased activation in the STS during the processing of the speech and simultaneous grooming trials, the activation in the STS was greatest during the presentation of gestures and speech. Holle at al. suggest that while this increased activation is not indicative of high-level semantic processing of gestural information, it is more likely a reflection of the matching of gesture (visual) to its associated segment of speech (audio). This response happens to a much lesser extent in the presence of the grooming visual stimulus, which did not interact with speech in a meaningful way. Holle et al. interpret the increased activation in the inferior parietal lobule and particularly the ventral premotor cortex as possible evidence of the mirror neuron system at work.

The mirror neuron system (which is covered extensively in Chapter 2), is thought to incorporate an array of cortical regions including the premotor cortex and

the inferior parietal lobule (Rizzolatti & Craighero, 2004), and has been attributed with assisting the understanding of goal-oriented actions (Iacoboni et al., 2005). In the case of gestural communication, Holle et al. (2008) suggest that the 'goal' that the mirror neuron system is working on in this study is determining "why did the speaker produce that hand movement?". In the case of grooming movements, the answer would be "because she wanted to scratch herself" and in the case of gesture, "because she wanted to show me what she means" (or "show me how the touching was done" in the mouse example) (Holle et al., 2008). If both of these actions require processing by the mirror neuron system, why is there increased activation during the gesture condition? Based on the notion that the mirror neuron system is part of a system that assists predicting the intended goals of observed actions (Iacoboni et al., 2005), Holle et al. suggest that the mirror neuron system has completed its task when the predicted goal of an action matches with the observed sensory input. If this initial prediction is incorrect and the matching process fails, then a new process of prediction commences, repeating cycles until a correct match is made. Holle et al. theorise that iconic gestures are more complex and deliberately more vague than grooming movements, increasing the number of possible goals for the action and therefore increasing the number simulation cycles that the mirror neuron system must process prior to arriving at a matching goal. Therefore they conclude that the increased activation seen in the premotor cortex and the inferior parietal lobule may be a reflection of the greater simulation requirements needed to process gestural information (Holle et al., 2008)

In another fMRI study Hubbard et al. (2009) examined brain activity while participants watched a video of a spontaneously produced speech stream while the presenter was gesturing, making nonsense hand movements, or holding their body

still. The gestures presented in the study were *beat* gestures, instead of the iconic co-speech gestures that much of this chapter has been devoted to (e.g., Holle at al., 2008). As mentioned earlier, beat gestures are the rhythmic gestures that do not necessarily semantically coincide with spoken word, but usually serve to punctuate the prosody of speech. The stimuli for this study was a series of video segments taken from a two-hour recording of an individual answering questions and spontaneously gesturing. The speaker's face was obscured, and segments of video were chosen that portrayed the speaker producing naturalistic non-iconic beat gestures. In the nonsense hand movement condition, the speaker produced non-iconic sign language in which they were paired with speech from the beat gesture condition. In the control condition in which the speaker held their body still, the video again was paired with speech from the beat gesture condition. As a further comparison, each condition was also matched with a silent condition in which participants only saw the visual stimuli and did not hear any speech. As expected, conditions in which speech was present led to increased activity in the STS and superior temporal gyrus (STG). Interestingly, greater activity was observed bilaterally in STS and STG when beat gestures accompanied speech compared to nonsense hand movements. Furthermore, greater activity was also observed in the planum temporale (PT) of the right hemisphere in response to beat gesture and speech than the sum of the activity of gesture or speech alone. Hubbard et al. theorise that right PT activity relates to the cortical synthesis of beat gestures and of prosodic speech information, as previous studies have shown that this region has been observed to be more active when listening to song melody versus speech, signing versus speaking, synchronized production of song lyrics, and processing pitch modulations (see Hubbard et al., 2009, for a review). Therefore, this increased

activity in the right PT suggests that this site is crucially involved in the integration of the rhythmic aspects of both speech and gesture (Hubbard et al., 2009).

While the above papers focus wholly on gesture comprehension, researchers have also used neuroimaging techniques to reveal the cortical regions associated with the production of gesture. Fridman et al. (2006) conducted an fMRI study in which participants read sentences instructed them to produce 'transitive' gestures (e.g., pretending to turn a doorknob or cut paper with scissors), 'intransitive' gestures (e.g., waving goodbye), or to produce no movement and to simply read. None of these conditions required participants to produce speech. In half of the trials participants were given the instruction "do it" following a delay period. Participants knew they were to perform the gesture only when reading this instruction. This allowed the researchers to identify cortical activity that arose from the preparation before an action as well as activity that occurred during the execution of the action. It was observed that during the preparation stages, increased activity was seen in the inferior frontal gyrus, the ventral premotor cortex, and the posterior parietal cortex. Executing the gestures led to greater activity in other areas such as the primary motor cortex and anterior intraparietal cortex. Though the authors do note that there are some differences in brain activity elicited by the two gesture types, as these results were fairly similar for both transitive and intransitive gestures. The primary difference between the two types appears to be increased activation in the ventral pre motor cortex during the preparation phase for transitive gestures, which the authors suggest is due to incorporation of an imagined 'tool' (e.g., scissors) when planning transitive gestures. Incorporating mirror neuron theory, the suggestion could be that the 'goal' of transitive gestures incorporates a specific object, therefore activating different areas of the proposed mirror neuron system.

In sum, the literature covered in this chapter strongly supports McNeill's (1985) theory that language and gesture are processed as part of the same underlying neural system. Through the research conducted over the decades since, that broad initial proposition could be refined to incorporate the knowledge that the relationship between speech and gesture is not only evident in behavioural observations, but also supported by recent psychophysiological data. The findings of the research discussed above indicate clear cortical links between both the production as well as the processing of gesture and speech information. In the case of iconic gestures, it has been demonstrated that the processing of gestural information is automatic (Holle & Gunter, 2007; Kelly et al., 2010), and can impact on processing at multiple time-points following the perception of speech (Kelly et al., 2004). It has also been demonstrated that gestures make it easier to process speech by reducing the cognitive load required for speech processing and help to disambiguate speech by adding extra information that is not available in the speech stream (Holle & Gunter, 2007; Holle et al. 2008). Furthermore, it has also been suggested that gestures are effectively goal-oriented actions, which is why areas associated with the mirror neuron system (specifically the premotor cortex) are more engaged when processing meaningful gestures (Holle et al., 2008).

Chapter 5: Transcranial Magnetic Stimulation

This chapter defines a method of cortical stimulation known as transcranial magnetic stimulation (TMS). TMS is used widely in research investigating cortical motor networks. There are many options for researchers using TMS protocols, and as such there are also a range of observed effects on physiology and behaviour. This chapter aims to explore the range of TMS applications, and the possible impact/importance this may have in research and clinical applications.

TMS is a method of non-invasive cortical stimulation that can be used to measure and modulate excitability of cortical regions. Based on Faraday's principles of electromagnetic induction, TMS involves the passing of an electrical current into an encased wire coil which generates a magnetic field. If the magnitude of this field changes, then it can induce a secondary current in a nearby conductor (Pascual-Leone, Walsh, & Rothwell, 2000). In the administration of TMS, the encased wire coil is positioned directly against the scalp, and a magnetic pulse radiates perpendicular from the coil penetrating the scalp and the skull, resulting in a current induction in local brain tissue. More specifically, the magnetic field induces an electric field in the brain causing ions to flow in the local cortical area. The flow of ions in the brain alters the electrical charge on both sides of neuronal cell membranes, leading to a depolarisation or hyperpolarisation of the neuron (Rossi, Hallett, Rossini, & Pascual-Leone, 2009). The change in polarisation triggers an event potential which activates associated corticospinal neurons transynaptically (Hallett, 2000). TMS coils primarily come in two different shapes, a standard round coil and figure-of-eight shaped coil which emits a magnetic field that is most powerful at the junction where the wire coils cross (Rothwell, 1997). A TMS pulse is

only believed to activate neurons at a depth of 1.5 cm to 2 cm (Wasserman, 1998). As the power of the magnetic pulse decreases greatly over a short distance from the coil, to ensure consistent and accurate pulse power, the focal area of the coil must be held directly against the scalp. TMS devices are designed so that the operator can adjust the output level (or 'intensity') of the TMS pulse, with greater intensities resulting in a larger, more powerful pulse (Riehl, 2008). A pulse of sufficient intensity causes a visible contraction in the target muscle, and also provides measurable changes in electromyographic (EMG) data recorded from the target muscles. A distinct waveform evoked by the TMS pulse that occurs within the EMG data recording is known as a motor-evoked potential (MEP). MEPs only occur as a direct result of the application of TMS over the motor cortex, and therefore are a reasonably stable and replicable indicator of cortical stimulation of regions of the brain that are responsible for controlling the muscle in which the MEP was recorded (Hallett, 2000). A proportionately large area of the motor cortex is made up of dedicated neurons that project to the hand muscles (Muir & Lemon, 1983) and the hand area of the motor cortex is often targeted by researchers looking at motor cortex function (e.g., Pascual-Leone at al. 1992, Chen et al. 1997). MEPs from hand muscles are often reported by researchers as hand MEPs can be evoked at low threshold. Wasserman (1998) suggests may be due to the "richness of the corticospinal projection that impinges on their spinal motor neurons" (p. 3).

Magnetic field penetration into the cortical substrate varies across individuals and therefore pulse intensity can be adjusted accordingly for each participant exposed to TMS (Riehl, 2008). Researchers can establish a minimum level intensity for motor areas when the target muscle is at rest – this is known as the resting motor threshold (RMT). RMT can be established individually for each participant, and is

usually calibrated based on desired pre-established MEP characteristics (e.g., Wasserman, 1998). The size of an electromagnetic pulse field discharged from the TMS coil is proportionate to the amount of area of the brain affected by the pulse (Schutter, Van Honk, & Panksepp, 2004). In other words, a low intensity pulse (though of an intensity sufficient to cross the skull and affect the neurons) can directly impact neurons below the focal point of the TMS coil, whereas higher intensity pulses recruit a more widely spread array of neurons. With the ability to stimulate focal target areas of the motor cortex and produce measurable responses in target muscles, TMS can therefore be used as a reliable method of mapping functional areas of the cortex with a spatial resolution of approximately 1cm (Schutter et al., 2004). TMS can be applied as a single stimulus, known as single-pulse TMS, in pairs of temporally discreet stimuli, known as paired-pulse TMS, or in trains of multiple stimuli known as repetitive TMS or (rTMS). Methodologies outlined in this thesis focus on the application of single-pulse and rTMS protocols and therefore these will be the focus of this review.

Single-Pulse TMS

In research involving the cortical motor networks, single-pulse TMS is widely used as a method of momentarily inducing activity in a distal muscle via stimulation of specific areas of the motor cortex. Single-pulse TMS has also been used to assess the task-specific functional importance of a brain region by transiently disrupting normal functioning in that specific cortical area.

The use of TMS in this fashion is commonly referred to in the literature as creating a 'virtual lesion' (Pascual-Leone et al., 2000). The basic premise of the virtual lesion experimental technique is the understanding that if a brain region is suspected of being involved in processing particular information or is recruited while

performing particular tasks, disrupting the functioning in that area of the brain will lead to a decrease in task performance (such as poorer accuracy or reaction time). For example, a number of researchers have used single-pulse TMS protocols to alter visual cortex function, and while TMS of the visual cortex logically produces no MEPs, a TMS pulse of sufficient intensity can cause a participant to perceive phosphenes in specific sections of their visual field, assisting researchers to identify appropriate pulse intensity (see Kammer, 2007 for a review). An example of such a study is that of Thielscher, Reichenbach, Uğurbil, and Uludağ (2010) who reported that applying single-pulse TMS over the occipital lobe 90 ms after the presentation of a visual stimulus reliably interfered with visual perception, reducing the participant's ability to accurately identify targets. Like Thielscher et al., many studies into TMS on the visual cortex have found that a TMS pulse delivered after the onset of a visual stimulus (usually between 80 ms to 130 ms) causes some momentary disruption to visual perception and a reduction in accurate visual identification (see Kammer, 2007 for a review). A distinct advantage of studies like this is that single-pulse TMS can offer insight into the chronometry (or timing) of the functional input of the target cortical area in cognitive processes (Pascual-Leone et al., 2000). Mulckhuyse, Kelley, Theeuwes, Walsh, and Lavie (2012) have extended research into the impacts of TMS on the visual cortex and report that in some cases it may also have a facilitatory effect on visual processing. In their study, Mulckhuyse et al. found that applying TMS 150 ms or 200 ms prior to stimulus onset actually improved performance on a visual perception task performance. While it is more difficult to glean chronometric information from a TMS pulse being delivered pre-stimulus, it does allow to researchers to speculate that the target brain area is somehow involved in preparing (or inhibiting) stimulus processing preparations.

The area of the brain most widely investigated using TMS techniques is the motor cortex (Jahanshahi & Rothwell, 2000). Pascual-Leone et al. (1992) examined the effect of single-pulse TMS on the motor cortex on reaction time. In their study, TMS was delivered over the region of the motor cortex responsible for controlling the biceps or the abductor pollicis brevis (APB) muscle in the contralateral arm/hand. Participants' response speed was measured in a series of simple go-signal reaction time tasks. Participants perceived the go signal visually, aurally or tactilely while TMS parameters were manipulated in order to investigate how reaction time might be altered by pulses delivered at differing intensities and at different latencies with reference to the go-signal. Reaction time was recorded as the onset of elbow flexion or thumb abduction. Single-pulse TMS was delivered at the onset of the stimulus and at ±5, 10, 20, 30, 40, and 50 ms either prior or post stimulus. Pascual-Leone et al. found that reaction time was faster than control when TMS was delivered between -10 ms and +20 ms of stimulus onset, and fastest at stimulus onset to 10 ms post stimulus onset (regardless of the modality of the go-signal). Reaction time was delayed when TMS was delivered at +50ms after stimulus onset. Pascual-Leone et al. also examined the impact of altering TMS pulse intensity. Using a 'sub-threshold' pulse intensity (defined as an highest intensity at which bicep MEPs were not present) as a baseline, Pascual-Leone et al. delivered TMS pulses time-locked to go-signal onset (0ms) and at intensities ranging from 80% to 125% of baseline. Compared to control reaction time, participants responded faster when the TMS pulse was delivered at 95-100% of baseline intensity and reaction time was delayed when TMS was delivered at 115%-125%. Pascual-Leone et al. conclude that faster reaction times are a result of magnetic stimulation reducing the time it takes for task-specific motor circuitry to initiate an action. In other words, single pulse TMS

delivered at a specific latency (-10 ms to +20 ms of stimulus onset) and at specific intensity (95% to 100% of subthreshold baseline) excites the neural pathways required for responding to the go-signal, leading to a decrease in reaction time.

Repetitive TMS (rTMS)

rTMS is the application of trains of TMS pulses to the brain and the effects of rTMS are quite different to those of single-pulse TMS. In addition to the central parameters of single-pulse protocol (such as pulse intensity, latency, coil type and location) researchers also have the ability to manipulate the frequency (or rate of) pulse discharge during rTMS, as well as the number of pulses that occur in a train, and the train frequency. The benefit of using rTMS over classic single-pulse TMS is that single-pulse only impacts brain activity at the moment of and shortly (approximately 100 - 200 ms) after the discharge of the magnetic pulse (Rothwell, 1997), whereas rTMS has the potential to change cortical excitability or disrupt function for a substantial period of time after stimulation. This extended period of cortical perturbation offers researchers more options for additional simultaneous physiological recordings and behavioural responses (e.g., Kobayashi, Hutchinson, Théoret, Schlaug, & Pascual-Leone, 2004)

An example of this is an early study conducted by Cohen et al. (1997), who examined the role that the visual cortex plays in Braille reading in congenitally (or early-) blind individuals. This was achieved by using rTMS to create a virtual lesion in the V1 area of the visual cortex while blind and sighted volunteers used their fingertips to read Braille and embossed Roman numerals. It was already previously known that the visual cortex of blind individuals was activated when Braille reading however Cohen et al. wished to know whether this was a result of cortical plasticity increasing tactile sensitivity (the visual cortex being recruited for tactile tasks,) or

the result of a heightened spatial discrimination ability. Cohen et al. found that disruption of functioning in V1 (via application of rTMS) led to an increase in reading errors, and distorted tactile perceptions of congenitally or early-blind participants, but did not impair sighted participants, or participants who were blinded at a later age. The authors concluded that if blindness occurs early enough, the visual cortex can be recruited to perform roles usually reserved for the somatosensory cortex such as the processing of tactile information.

Beyond virtual lesions, rTMS allows researchers to create changes in cortical excitability and in order to provide physiological evidence for the depression or conversely, excitation of cortical networks stimulated by rTMS. Measuring cortical excitability assists researchers to understand changes in brain physiology or overt behaviour that may be as a result of brain disorders or deliberate manipulations of cortical plasticity (Hallett, 2000). One of the most widely used measures of change in cortical excitability in TMS is changes in MEP recordings. As mentioned above, an MEP is a unique waveform seen in EMG data which occurs shortly (approximately 20 ms for hand muscles) after a TMS pulse of sufficient intensity is delivered to the motor cortex. As this waveform occurs as a direct result of the application of a TMS pulse, and as an MEP of sufficient size is usually distinguishable from other motor activity due to its unique amplitude and waveform pattern. Researchers are able measure characteristics of the MEP (such as peak amplitude or waveform area) to assess excitability, and ultimately assess changes in excitability through MEP data at different time points (such as before and after a therapeutic treatment or experimental manipulation).

The use of rTMS as a method to effect cortical changes in excitability has been demonstrated by many authors including Pascual-Leone et al. (1993). Pascual-

Leone et al. found that low frequency and intensity rTMS delivered focally over the hand area of the motor cortex produced a series of similar MEPs in the hand muscles. As intensity and frequency were increased, MEPs spread to other muscles in the arm, or more specifically to areas that were somatopically promixal to the hand area of the motor cortex. It was noted that as the effect of the rTMS spread beyond the focal area, the latency at which the MEPs were evoked was longer for muscles further away from the hand. Wasserman (1998) suggests this is indicative of activation of increasingly distant or higher threshold areas being activated though intracortical induction.

There are a wide range of pulse repetition frequencies used in the literature, leading to a distinction between low frequency rTMS, which applies to rTMS pulse frequency of 1 Hz or slower, and high frequency rTMS which are delivered at a rate faster than 1 Hz (Rossi et al., 2009). Other authors view high frequency rTMS somewhat differently and suggest that high frequency is the label applied to rates of 5 Hz or greater (Khedr, Rothwell, Ahmed, Shawky, & Farouk, 2007). Regardless of disagreement over definition, many authors agree that as a general rule high frequency rTMS is likely to lead to facilitative effects on cortical excitability, whereas low frequency and irregularly patterned rTMS protocols are more likely to lead to inhibitory effects (Gangitano et al., 2002; Khedr et al., 2007; Rossi et al., 2009).

An example of low frequency rTMS creating inhibition can be seen in research by Chen et al. (1997). By measuring changes in MEP amplitude from the right APB, biceps, and deltoid muscles, Chen et al. found that although an hour of TMS pulses at 0.1 Hz did not lead to any changes in cortical excitability, 15 minutes of rTMS at 0.9 Hz led to a decrease in MEP amplitude which persisted for

approximately 15 minutes post-stimulation. Regarding high frequency rTMS, Khedr et al. (2007) applied twelve trains of 25 Hz rTMS. Each train was 5 seconds long with an intertrain interval of 10 (total 1500 pulses) and pulse intensity was configured to 90% to 100% of RMT. Khedr et al. report that this protocol led to an overall increase in motor cortex excitability, which persisted for up to 30 minutes post stimulation. Other researchers have tested inhibition and facilitation in the same subjects. Gangitano et al. (2002) applied rTMS at 90% of RMT at a rate of 1 Hz or 20 Hz on two separate occasions. In the 1 Hz condition, 1600 pulses were delivered regularly over a 26 minute period, whereas the 20 Hz rTMS was delivered 40 trains of 40 pulses (lasting 2 seconds each) at intervals of 28 seconds. With MEP characteristics averaged across all 16 participants, the researchers found that a 1 Hz protocol led to a decrease in cortical excitability whereas a 20 Hz protocol led to an increase in cortical excitability. The results from these studies are by no means presented in isolation. Multiple other publications also indicated inhibition and facilitation effects of low and high frequency rTMS protocols in normal volunteers (e.g., Kim, Park, Ko, Jang, & Lee, 2004; Romero, Anschel, Sparing, Gangitano & Pascual-Leone, 2002) as well as clinical populations, such as a Parkinson's Disease sample (Lefaucheur, et al., 2004)

Some researchers have noted the specific challenges borne by the inconsistency of inhibitory/excitability effects of rTMS protocols across multiple participants (Maeda, Keenan, Tormos, Topka, Pascual-Leone, 2000). In fact, in the Gangitano et al. (2002) study, despite data averaged across the sample indicating low frequency rTMS creating cortical inhibition and high frequency creating facilitation, the authors note there was a small subset of their participants (4 of 16) whose results indicated the opposite effect. To investigate inter-individual differences further

Maeda et al. applied trains of 240 pulses at 90% RMT to the motor cortex at a ranges of frequencies, namely 1 Hz, 10 Hz, 15 Hz, and 20 Hz. While they found that average excitability across participants increased with frequency, the excitability patterns were very heterogeneous. A graphed representation of excitability provided by Maeda et al. (measured as percentage change in MEP area) shows large variability between participants, with some participants not demonstrating the expected linear increase in excitability across frequencies. In a second experiment, the same authors increased the number of pulses to 1600, and tested participants at 1 Hz and 10 Hz. They found that this group was much more homogenous with most participants displaying a similar increase in excitability between to two frequencies (Maeda et al., 2000). While the authors suggest limitations in their study regarding the number/frequency of pulses and resting periods between conditions, they do attribute inter-individual variability in excitability patterns to neurophysiological differences between individuals (Maeda et al., 2000). From results such as these, it could therefore be suggested that the effects of rTMS are not universal and researchers must keep this in mind when developing rTMS protocols, particularly when investigating specific effects of rTMS on cortical excitability.

In a more recent study investigating rTMS responder variability, Daskalakis, et al. (2006) applied 900 pulses of rTMS at 90% of RMT at frequencies of 1 Hz, 10 Hz, and 20 Hz. Daskalakis et al. were interested in measuring potential changes in both facilitation and inhibition following stimulation, and examining the possible relationship between excitability and pre-stimulation MEP characteristics. Although the authors found no overall changes in inhibition measures at any frequency, they did find that participants who demonstrated lower inhibition in baseline testing were likely to display greater cortical inhibition following rTMS. Daskalakis et al.

interpret this as potential evidence of homeostatic plasticity (i.e., neural influences keeping excitability within a normal physiological range) and that the increased inhibition following rTMS seen in these participants may be a reflection of neural mechanisms homeostatically compensating for potential excitability changes caused by rTMS. The authors conclude that future studies could use baseline physiological measures to determine potential likelihood of responsiveness to rTMS techniques.

Huang, Edwards, Rounis, Bhatia, and Rothwell (2005) report on a specific rTMS protocol known as theta-burst stimulation (TBS). TBS is characterised by single short low intensity 20 Hz bursts of TMS pulses (Huang et al., 2005). The author studied the effects of three distinct TBS patterns, each totalling 600 pulses (in 20 Hz bursts of three pulses delivered at 50 Hz intervals) over the motor cortex. Specifically, Huang et al. targeted stimulation at the region of the motor cortex responsible for control of hand muscles, and recorded EMG data from the first dorsal interosseous (FDI) muscle in the contralateral hand. Stimulator intensity was set at 80% of active motor threshold (AMT), which Huang et al. calibrated as the threshold for distinct MEPs while participants maintained a voluntary muscle contraction approximating 20% of maximal force. These patterns were continuous TBS (cTBS), consisting of 20 seconds of continuous bursts, intermittent TBS (iTBS), consisting of 2 second-long trains of pulse bursts every 10 seconds, and intermediate TBS (imTBS) consisting of 5 second-long trains every 15 seconds. Compared to baseline excitability, imTBS evoked no change in excitability, however, cTBS led to motor cortex inhibition and iTBS led to increases in excitability – effects which lasted for approximately 20 minutes post-stimulation (Huang et al., 2005). In a follow-up experiment, Huang et al. investigated whether the length of the initial stimulation would impact the longevity of the changes to cortical excitability. The authors found

that doubling the period of cTBS stimulation to 40 seconds led to a comparative increase in the period of inhibition, as cortical excitability was reduced for up to 60 minutes. Although these findings are only based on data gathered from nine individual subjects, Huang et al. propose cTBS as a reliable protocol for depressing motor cortex excitability. In a follow up study, Martin, Gandevia, and Taylor (2006) examined the efficacy of cTBS on excitability in different muscle groups. In that study, 40 seconds of cTBS was applied to the two areas of the motor cortex that produced maximal MEPs for both the contralateral FDI and biceps muscles. The authors report that FDI MEP amplitude was significantly depressed for up to 30 minutes post cTBS when the FDI cortical region was targeted. However, when the biceps motor-region was stimulated, bicep MEPs did not show reliable consistent depression. From these results, Martin et al. conclude that the reliability of cTBS varies between cortical regions, and observations of a distinct depression in cortical excitability in one section of the motor cortex cannot necessarily be extended to other motor cortical areas.

A very recent study by Vernet et al. (in press) examines the reliability of cTBS effects over multiple sessions. Data were gathered retrospectively from 10 participants across two sessions separated by an average of 107 (±55) days. cTBS was administered to participants in both sessions and measures of pre and post stimulation excitability (at 5 min, 10 min, 20 min and 30 min post cTBS) were recorded. Vernet et al. report high intra-individual variability in excitability and depression between the two sessions. Variability was seen across all the measured time-points, however measurements at the post-5 minute time-point showed the least variability. As data were collated retrospectively, the methodological controls were not ideal. The authors note that there are extraneous variables not accounted for that

may influence excitability such as physiological brain state at the time of testing (Vernet et al., in press), and it is also important to note that there was inconsistency in testing procedure as well as a variable time period between test sessions. Vernet et al. report they are one of the first research groups to perform a study investigating intra-individual excitability variation across cTBS sessions, and despite some limitations, these results indicate that cTBS-induced cortical depression may not be consistent within an individual across multiple applications.

Applications of rTMS in Research and Clinical Treatment

Beyond simply investigating changes in cortical excitability, rTMS can be used also create behavioural change. In an early rTMS study, Pascual-Leone, Gates, and Dhuna (1991) demonstrated that rTMS could be used to produce selective interruptions to neural activity by targeting areas involved in speech production when applied over the language dominant hemisphere. The six epileptic participants in the study were eligible for surgical treatment, and therefore also underwent an intracarotid amobarbital (Wada) test; an established, but somewhat invasive test of hemispheric language dominance. Pascual-Leone et al. were able to compare the results of this test with the hemispheric location of rTMS which evoked speech arrest during a counting task. Although Pascual-Leone et al. report that they cannot precisely know which cortical area was being stimulated, rTMS was administered bilaterally over frontal regions approximating the motor cortex (and perhaps Broca's area in the left hemisphere). The authors found that in all six participants, the Wada test indicated left hemisphere language dominance, paralleling the finding that rTMS of the left hemisphere led to speech arrest. In further studies by Jennum, Friberg, Fuglsang-Frederiksen, and Dam (1994) and Epstein, et al. (1996), researchers went on to use this transient disruption of speech motor areas to develop a reliable and

safe method of inducing speech arrest in normal participants. Researchers found that rTMS only induced speech arrest in participants when magnetic stimulation was applied specifically to the speech motor area of their language dominant hemisphere (usually the left), and therefore suggested that rTMS could be used as practical and non-invasive (although sometimes uncomfortable) method of determining hemispheric dominance for language (Epstein et al., 1994)

As noted, MEPs are only evoked when TMS is applied to motor cortical structures, and would not arise if targeting other areas, such as Broca's area. Assessing TMS induced neural changes in non-motor areas relies on convergent evidence (e.g., behavioural recordings or imaging data). Aziz-Zadeh, Cattaneo, Rochat, and Rizzolatti (2005) applied rTMS over a number of different sites in both the left and right cerebral hemispheres. In both hemispheres, the inferior frontal gyrus (approximating Broca's area in the left hemisphere) and the motor cortex area responsible for activating muscles in the hand (opponens pollicis) and lips (mentalis). Aziz-Adeh et al. aimed to create two distinct effects with the rTMS. Firstly, they aimed to interrupt function of the mentalis and surrounding muscles, thereby inducing motor speech arrest, and secondly by stimulating Broca's area, they aimed to evoke non-motor speech arrest. It should be noted that a difference between this study and those previously reported is that the researchers were not aiming to create cortical changes that lasted beyond the duration of rTMS, and instead were only focused on direct behavioural changes that were observable during the active application period. To evoke motor speech arrest, rTMS of incrementally increasing intensity was applied over the motor cortex in short trains of 10 pulses at 5 Hz while participants counted aloud from 1 to 10. The rTMS protocol continued until speech arrest was observed or participants reported pain. A similar rTMS protocol was

applied to Broca's area and again, stimulation ceased when speech arrest was observable. Once Aziz-Zadeh et al. had determined the threshold for speech arrest, participants were then required to complete a syllable counting task to assess their overt and covert speech performance. Various multi-syllable words were presented on screen and participants were required to count the number of syllables either out loud (overt) or silently (covert) and then respond via a button-press. Aziz-Zadeh et al. found that when the left hemisphere sites (both motor and Broca's areas) were stimulated, reaction times were worse in both the overt and covert speech tasks. However in the right hemisphere, overt speech was only affected by stimulation of the motor site, and covert speech was not affected by stimulation of motor or non-motor (right inferior frontal gyrus) sites. The authors not only interpret this as evidence that rTMS can create reliable dysfunction in Broca's area, but also that Broca's and the mouth areas of the motor cortex are involved in cognitive language processes.

Behavioural observations are not just limited to inhibition rTMS protocols; some researchers have utilised the facilitative effects of rTMS to improve behavioural responses. Mottaghy et al. (1999) applied 2 second long 20 Hz rTMS over Broca's and Wernicke's areas and their right hemisphere homologues, and bilaterally in the primary visual cortex. Directly after stimulation of each site participants were tasked with rapidly naming common objects appearing on a monitor, before the coil was relocated to another site for further stimulation. Unlike protocols previously covered in which intensity is calibrated according to individual participant threshold, Mottaghy et al. set rTMS intensity at 55% of maximal stimulator output as they report it was too difficult to determine threshold at these stimulation sites. Mottaghy et al. report that while rTMS had no impact on accuracy,

when applied over Wernicke's area, rTMS led to reduced picture naming latencies. This effect was most prominent directly after stimulation, but reduced at 30 seconds post-stimulation, and was entirely extinguished at 2 minutes. Furthermore, this effect was not seen following stimulation at any other site. Despite reservations about the accuracy of the coil position and the underlying cortical structures, the authors concluded that these results imply Wernicke's area involvement in picture naming, and also that rTMS can be used to facilitate cortical function, if only for a very brief period of time.

Since the mid-1990s, rTMS protocols have also been adapted so that they could be trialled in therapeutic recovery for a number of psychiatric and neurological disorders (Wassermann & Lisanby, 2001). The basic premise of the therapeutic application of rTMS is that patients adhere to a treatment regimen in which stimulation is applied to target cortical areas on a regular basis. For example, in the treatment of mood disorders, Speer et al. (2000) compared the effect of daily treatments of low or high frequency rTMS over the left prefrontal cortex on ten participants diagnosed with depression. Participants were given ten daily sessions of each rTMS frequency (20 days total) and mood assessments and PET scans were conducted at baseline and throughout treatment. Speer et al. found that high frequency (20 Hz) rTMS let to increases in cerebral blood flow in the left prefrontal cortex, as well as the left cingulate gyrus and amygdala, and bilaterally in the insula, basal ganglia, hippocampus, thalamus, and cerebellum. Furthermore, Speer et al. report that participants reported an improvement in mood following the high frequency rTMS, and a decline in mood following low frequency rTMS treatment. A number of important implications can be identified in these results. Firstly, observation of the impacts of rTMS are not restricted to just to behavioural

observations, and the convergent physiological evidence supports the notion of frequency-specific impacts of rTMS. Secondly, the repeated application of rTMS over one focal area leads to blood flow changes in other cortical areas, a clear demonstration that long term rTMS does not only impact the target area, but can effect change in broader cerebral networks. Thirdly, the effects of regular rTMS appear cumulative in effecting change, and finally, findings such as these also implicate the utilisation of rTMS beyond research and into treatment and rehabilitation.

While Speer et al. (2000) only assessed participants over a short duration, other authors have reported that rTMS is also effective in relieving depression symptoms when maintained over a longer period (O'Reardon, Blumner, Peshek, Pradilla,& Pimiento, 2005) . O'Reardon et al. maintained high frequency (10 Hz) rTMS treatments once or twice weekly over a 6 month- to 6 year-period. Seven of the ten participants in this study experienced improvement during the course of treatment, which was maintained without the addition of medication in three cases. Authors such as Loo and Mitchell (2005) argued that the utilisation of rTMS protocols in the treatment of psychiatric disorders is in need of further exploration and refinement, and the small sample size of O'Reardon et al. and unavoidable possibility of spontaneous recovery over time make results like these difficult to generalise. Despite this, more recent findings from a meta-analytic study indicate that rTMS is a valid anti-depressant technique (Gross, Nakamura, Pascual-Leone, & Frengi, 2007), so much so that rTMS is an approved treatment for depression in both the United States and Canada (Kennedy et al. 2009).

As mentioned above, research into the therapeutic application of rTMS does not just provide more insight into the impacts of regular TMS application, but also

demonstrates the utilisation of physiological evidence to support behavioural observation. The effects of rTMS can also be reflected in physiological information from other methodologies such as combined TMS-EEG studies. Hansenne, Laloyaux, Mardaga, and Ansseau (2004) and Laloyaux, Ansseau, and Hansenne (2006) applied low and high-frequency rTMS over the pre-frontal cortex in order to assess changes in stimuli processing. Hansenne et al., examined changes in the ERP component P300, which they state is a positive component occurring approximately 300 ms post stimuli, and that it is an indicator of the participant detecting task relevant stimuli. Inhibition and automatic processing were indexed via the mismatched-negativity (MMN) ERP peak which can be identified as a negative amplitude component occurring between 100 and 250 ms post stimulus and can be evoked when participants hear a sound that deviates from a prior sound sequence (Laloyaux et al., 2006). The authors report that rTMS evoked no change to the amplitude of latency of MMN component (Laloyaux et al., 2006) or P300, but that rTMS did delay latency of the P300 component (Hansenne et al., 2004). Combining these findings, Laloyaux et al. suggest that this is evidence that rTMS impacts controlled processes (P300) as opposed to automatic processes (indexed by MMN) when applied to the prefrontal cortex. Hansenne et al. conclude that rTMS in this area only affects the speed of processing. These studies provide good examples of beneficial outcomes from combined rTMS and EEG methodologies.

Neural Compensation and Non-focal TMS Effects

More recently, advances in imaging techniques have allowed researchers to examine cortical changes broader than just those in the focal target area. In a review article, Ruff, Driver, and Bestmann (2009) highlight a number of published studies which have found diffuse cortical impacts of TMS that are reflected in fMRI data.

For example, Denslow, Lomarev, George, and Bohning (2005) found that after low frequency rTMS was applied over the primary motor cortex, fMRI data showed modulations in activity throughout broader cortical motor networks including the premotor cortex and supplementary motor areas. These ‘network-wide’ impacts of TMS are not restricted to motor systems, and can be observed in other cortical areas. For example, stimulation applied over the frontal-eye field (located in the prefrontal cortex) can effect activity change in primary visual cortical areas located in the occipital lobe (Ruff et al., 2006).

If rTMS in a focal area can lead to temporary network-wide changes (e.g., Ruff et al., 2006; Speer et al., 2000) perhaps it would be scientifically advantageous to acknowledge the possible functional impact of rTMS on non-focal areas. In 2008, Andoh and Martinot hypothesised that "during interference with a local brain area, rTMS could induce a functional reorganisation in remote areas homologous to the 'virtual' lesion to compensate the stimulated and disturbed area" (p.285). Kobayashi et al. (2004) report that following low frequency rTMS (1 Hz) over the hand area of the primary motor cortex during a finger tapping task, they found that while task performance was unchanged in the contralateral hand, reaction time improved in the ipsilateral hand. Kobayashi et al. theorise that this is due to 'transcollosal inhibition' emanating from the target motor cortex, which effectively leads to a "release of excitability for the unstimulated contralateral motor cortex" (p. 96), and ultimately faster reaction times for the hand controlled by that unstimulated area. Andoh and Martinot define effects such as this as 'interhemispheric compensation', suggesting the homologous compensatory area would most likely be located the contralateral hemisphere to that of the stimulated area. Andoh and Martinot also review interhemispheric compensation in non-motor cortical networks, and reflect on the

impact of rTMS on other cortical functions such as language processing. For example, in a PET study, Thiel et al. (2006), inhibited the left inferior gyrus (Broca's area) using rTMS and concurrently examined cerebral blood flow while participants performed a verb generation task. The authors report that compared to control, rTMS not only increased reaction time in the task, but also lead to decreased blood flow in the left inferior frontal gyrus, while increasing it in the unstimulated right inferior frontal gyrus. Thiel et al. conclude this is evidence of transcallosal inhibition, and propose that in this case, the suppression of the language-dominant left hemisphere Broca's area led to increases in metabolic activity in the right hemisphere homologue, suggesting a temporary rTMS induced rightward movement of linguistic function.

Distinct from the temporally restricted (during active stimulation only) interhemispheric changes reported by Thiel et al. (2006), Andoh and Martinot (2008) hypothesise that repeated long-term therapeutic application of rTMS may induce more long lasting interhemispheric changes. Andoh and Martinot focus on the use of rTMS to inhibit Wernicke's area in order to reduce or inhibit auditory hallucinations in patients with schizophrenia. In this population, auditory hallucinations are the thought to be the result of a malfunctioning region (see Andoh and Martinot, 2008, for a review), and successful long-term treatment of auditory hallucination using rTMS is ultimately due to sustained regular inhibition of this malfunctioning Wernicke's area. Andoh and Martinot hypothesise that suppression of the dominant left hemisphere Wernicke's area, leads to an increase in activity in the right hemisphere homologue (superior temporal gyrus and surrounds), which presumably functions without the appearance of symptomatic hallucinations. While only a theoretical proposal, to support their hypothesis the authors cite evidence from PET

studies in which *actual* brain lesions (as opposed to rTMS induced virtual lesions) have led to increased blood flow in the right Wernicke's homologue during a comprehension task compared to rest (Musso et al., 1999). Andoh and Martinot conclude that while they cannot be certain of the array of neural changes that accompany rTMS induced changes in auditory hallucinations, they cannot discount the potential impact of interhemispeheric compensation mechanisms.

In a very recent study Zanto, Chadick, Satris, and Gazzaley (2013) suggest that these system-wide effects of rTMS may actually represent a stimulation-induced rapid reorganisation of cortical function, in other words, non-target cortical areas are able to adapt within a short post-stimulation time period, and may even provide compensatory function for the part 'lesioned' brain area. Twenty two participants performed a visual working memory task after rTMS had been applied to inhibit function in the right inferior frontal junction (IFJ). In this task, participants looked at overlayed images of landscapes and faces and were required to attend to and memorise target stimuli. Using fMRI data, Zanto et al. were able to examine changes in activity that occurred post-stimulation while participants viewed, memorised and recalled stimuli. In an earlier study, Zanto, Rubens, Thangavel, and Gazzaley (2011) found that rTMS over the right IFJ had impaired accuracy in a task in which participants were required to attend to/remember movement and colour information from a dot-array. Despite the overall performance decline following rTMS, Zanto et al. (2011) report pronounced variability between participants. In the recent and more advanced task, Zanto et al. (2013) hypothesised that when rTMS inhibits the IFJ, performance would be impaired and memory accuracy would decrease. While Zanto et al. (2013) report reduced IFJ function following rTMS, despite their predictions, they also found task accuracy and reaction time were unchanged by the

administration of rTMS compared to control. Interestingly, Zanto et al. (2013) found that when rTMS was administered, increased activity was observed in a remote region of the brain, specifically the left lateral occipital complex (LOC). Although the left LOC is not the right-hemisphere homologue of the IFJ, Zanto et al. (2013) hypothesised that the increased activity seen in the left LOC following rTMS of the right IFJ was the result of compensatory neural activity, allowing the brain to process stimuli despite IFJ inhibition. Accordingly, Zanto et al. (2013) re-examined behavioural data allowing for variance in LOC activity, and found that while accuracy was still unchanged, participants who showed greater activity in LOC had faster reaction times than those with less LOC involvement. Zanto et al. (2013) conclude that LOC activity was representative of compensatory neural activity, which rapidly occurred post-rTMS administration, allowing faster processing than when compensation did not occur (or occurred to a lesser degree). It is important to note the rapidity at which this compensatory change occurred. It did not occur over multiple sessions (as suggested by Andoh & Martinot, 2008), but happened within minutes of rTMS administration, which Zanto et al. (2013) identify as "rapid functional reorganisation" (p.16268).

In summary, the TMS literature contains many examples of the use of rTMS to effectively excite or inhibit activity in a variety of distinct cortical regions, including the motor and linguistic cortical areas (Aziz-Zadeh et al., 2005; Epstein et al., 1996; Huang et al., 2005; Jennum et al., 1994; Martin et al., 2006; Pascal-Leone et al., 1991), as well as other frontal lobe regions (Hansenne et al. 2004; Laloyaux et al., 2006). The effects of rTMS are seen to last beyond the duration of active administration and can persist for a number of minutes following administration (e.g., Huang et al., 2005), or across a broader temporal scale when administered over

multiple sessions (e.g., for the treatment of psychiatric disorders) (Gross et al., 2007; Loo & Mitchell, 2005; O'Reardon et al., 2005; Speer et al., 2000). Using imaging data and EEG other authors have sought to find convergent evidence of the neural changes elicited by rTMS, and the broader impacts in may have on interconnected neural systems (e.g., Denslow et al., 2005; Laloyaux et al., 2006; Ruff et al., 2005). Reports of neural activity changing in sites distinct from the rTMS target area led some authors to propose that these other cortical areas may be displaying increased activation due to the suppression of the usually dominant target area (Thiel et al., 2006), and homologous areas may show increased activation in order to compensate for the impaired functionality (Andoh & Martinot, 2008). Furthermore, Zanto et al. (2013) have proposed that these compensatory functions are not necessarily restricted to direct hemispheric homologues, and may rapidly occur within minutes of administration.

Chapter 6: Study One

Introduction

There are a number of elegant studies that have been developed over the past two decades investigating links between the motor cortex and language, and although they are reviewed more comprehensively elsewhere in this thesis, below is a brief overview. Results from neuroimaging studies demonstrate increased activity in the areas of the motor cortex when processing action-related language. Results from fMRI studies indicate overlapping activation patterns in the effector-specific regions in the motor cortex when reading action verbs and performing the described action (Hauk et al., 2004) or when reading phrases that included a specific effector and observing that action (Aziz-Zadeh et al., 2006). Even without the comparison of action observation, neuroimaging researchers have reported increased motor cortex activity when listening to sentences that describe actions compared to abstract sentence (Tettamanti et al., 2005), or when participants perceived action words (nouns or verbs) compared to non-action words (Vigliocco et al., 2006).

The notion that the motor cortex in involved in processing motor-related language has led some researchers to investigate how motor cortex activity could be measured using TMS – a technique used widely in motor control research in which researchers induce EMG activity data and interpret it as reflection of motor cortex cortical activity. Buccino et al. (2005) used TMS to compare MEP (a specific component of EMG) amplitudes when listening to either hand-based or foot-based action sentences, or abstract sentences. Buccino et al. found that when listening to a hand-based action sentence, MEPs were significantly lower in the hand muscles compared to foot-based action and abstract sentences. Similarly, MEPs were

significantly lower in the foot muscles when listening to foot-based action sentences compared with the other two conditions. Based on the notion that larger MEPs are indicative of higher excitability of the corticospinal pathway and smaller MEPs indicate lower excitability (Rothwell, 1997), the researchers interpret these excitability fluctuations as evidence that the motor cortex is involved in action-language processing. However, they acknowledge that it would be expected that there would have been an increase (rather than the observed decrease) in MEP amplitude as demonstrated in other action observation studies (see Buccino et al., 2005, for a review).

Oliveri et al. (2004a) conducted a series of TMS studies investigating the role of motor cortical regions on the processing of action-related words. In one of these studies, participants were required to produce utterances comprising of action verbs (e.g., '*to strangle*'), action nouns (e.g., '*the axe*'), non-action verbs (e.g., '*to adore*'), and non-action nouns (e.g., '*the carpet*'). Using paired-pulse TMS, Oliveri et al. assessed changes in motor cortex excitability when participants produced a list of utterances including these different word types. Paired-pulse methodology generally consists of administration of a subthreshold conditioning stimulus followed by a suprathreshold test stimulus. Based on the time between pulses and variations in MEP size, researchers are able to infer changes in the excitability of intracortical inhibitory and facilitatory circuits (Oliveri et al., 2004a). Measuring MEP size, the results showed that when TMS was applied over the hand-area of the motor cortex, there was a significant increase in excitability when producing motor related words compared to non-motor words. Interestingly, Oliveri et al. report that this effect transcends grammatical class division as increased excitability was observed in both action-noun and action-verbs, and there was no significant difference between word

classes within either action-type. The authors cite this as evidence that the motor cortex is stimulated by more than just description of an executable action, and that processing words that merely describe an object that would be involved in an action also recruits the motor cortex. While Oliveri et al. do not draw a direct connection to mirror neuron activity, these findings fit within a broader mirror neuron framework and are concurrent with embodied cognition theories (Gallese & Lakoff, 2005) and the links between the motor cortex and imagining object-specific actions.

While Oliveri et al. (2004a) state that they wouldn't necessarily expect similar action versus non-action results using single-pulse TMS methodology, other researchers have found that single-pulse stimulation can also produce facilitative differences in reaction time (RT) to action words, depending on the region of the cortex stimulated, and the effector being used in the described action. As covered in the TMS chapter of this thesis (Chapter 5), single-pulse TMS can be applied during a task with the intention of impacting stimulus processing and change behavioural outcome. Pulvermüller et al. (2005) set out to investigate the impact of applying single-pulse TMS to the motor cortex of participants while they viewed string of words consisting of arm actions, leg actions, distracter words and pseudowords. A TMS pulse was delivered at 90% of resting motor threshold (RMT) over the left motor cortex 150 ms after the presentation of each item while EMG data were recorded from a muscle in the contralateral forearm as well as the leg. The choice of delivering the TMS pulse at 150 ms in this study is based on previous reports that the time period during which semantic processing is thought to start is 100 to 200 ms (Pulvermüller, 2001; Skrandies, 1998). The pulse was delivered at an intensity of 90% of the RMT with the expectation that this would prime motor cortex functioning and therefore facilitate language processing in that area of the brain.

Participants were asked to respond to meaningful words by making a brisk mouth movement and accuracy and RT was calculated for each type of word. Pulvermüller et al. observed faster RT to arm action words compared to leg action words after TMS of the area of motor cortex responsible for the arm, and vice versa for TMS of the area of the motor cortex responsible for the leg. This finding was interpreted as evidence that words that are semantically linked to hand or leg actions are processed by the effector-specific area of the motor cortex responsible for controlling those limbs.

Based on an adaptation of the Pulvermüller et al. (2005) methodology, in a previous study (Mertens, 2009), I set out to investigate the effect of a supra-threshold TMS pulse on the processing of hand-action words in the motor cortex. In that study, participants viewed a list of hand-action and non-action (or 'static') verbs while single pulse TMS was applied to the hand area of the motor cortex. Based on the findings that a sub-threshold TMS pulse applied at 150 ms facilitates the processing of motor-action verbs (Pulvermüller et al., 2005), and previous reports of supra-threshold TMS delaying reaction time (Pascual-Leone et al., 1992) I wished to determine if a supra-threshold TMS pulse of 120% of RMT applied to the hand area of the motor cortex would inhibit processing of hand-action verbs, and therefore delay reaction time (RT). In that study, 15 participants viewed a list of words that consisted of a mix of hand-action verbs (such as *wipe,* or *carry*), static verbs (such as *dream,* or *fear*), and pseudowords which were used as distracters. Participants were required to produce a quick jaw-clench response only upon viewing a real English word, and differences in RT between TMS and sham (control) trials were analysed. Although RT was significantly slower for action-verbs, application of TMS yielded no significant change to RT for either type of word. It could therefore be inferred

that as a TMS pulse delivered to the hand-area of the motor cortex at 120% of RMT at 150 ms post stimulus onset does not create any discernible delay in RT, this protocol does not cause inhibition in the processing of hand-action words. Based on these results, I was unable to conclusively rule out the notion that supra-threshold TMS causing inhibition to motor-cortex processing of motor-related words, but instead concluded that the lack of findings in this study may be due to the pulse latency not targeting the appropriate stage of language processing to produce inhibitory effects. Furthermore, I also suggested that the facilitatory effect found by Pulvermüller et al. was specific to the application of a pulse of 90% of RMT delivered at 150 ms post stimulus onset.

The current study aims to further elaborate on the findings of my earlier study (Mertens, 2009) by investigating the effects of single pulse TMS applied over the hand-area of the motor cortex at a range of different intensities and latencies. To determine an appropriate range of pulse latencies, it is necessary to further explore the literature examining the time course of language processing throughout linguistic cortical networks. While not investigating neural activity in response to motor-related words, Skrandies (1998) examined ERP component divergence due to semantic differentiation. In that study, EEG data were recorded from participants who viewed 60 German nouns that had been previously rated as 'high' on one aspect of one of three dimensions; *evaluation* (good-bad); *potency* (strong-weak); and *activity* (active-passive). In order to ensure participants were attending, they were asked to actively remember and visualise the words presented as their memory would later tested. Skrandies specifically investigated the P100 ERP component - a positive component occurring around 100 ms post stimulus onset. It was observed that compared to control (viewing a checkboard pattern), word stimuli elicited a

delayed P100 peak latency (by approximately 4 ms). Furthermore, words that ranked high or low on the *activity* dimension elicited later P100 peaks, compared to words ranked moderately on this scale. Skrandies also observed changes in other sections of the ERP waveform and found that words high or low in the *potency* dimension elicited greater amplitudes of a negative component at approximately 160 ms, and a delayed peak latency for a negative component occurring at approximately 220 ms. While Skrandies also reports some further distinctions in later components, he concluded that most of the significant early semantic effects were observed at around the latency of 100 ms. Skrandies admits this latency is early compared to other studies which report semantic processing occurring much later (such as the N400 reported by Kutas and Hillyard [1980]). While he cautions against interpreting reading rate studies alongside his electrophysiological results, Skrandies explains that early semantic processing is quite possible as there is evidence that the human brain can rapidly process words when speed reading (as Rubin and Turano [1992] report that some participants can read at up to 30 words per second).

As indicated by Skrandies (1992) and outlined earlier in this thesis, EEG studies have previously identified the much later N400 component as reflective of processing linguistic information (for a review see Kutas & Federmeier, 2011). In many studies the N400 peak appears to be attenuated by attempts to integrate semantic information from a linguistic stimulus. Modulations in N400 amplitude can be driven by a participant's expectations of a stimulus, or more accurately, the incongruency of the semantic link between a priming stimulus and the subsequent target stimulus in both word pairs and sentences (Kutas, 1993). In a comprehensive review of N400 literature, Kutas and Federmeier are cautious to point out that despite the wide investigation of the N400 in language studies, the N400 is not solely

linked to pure linguistic processing and modulations in N400 can be seen in studies that use a broad range of less linguistically salient stimuli (such as pictures, or actions) and can also be used to examine aspects of semantic memory (Kutas & Federmeier, 2011).

In a study more relevant to the time-course of processing motor-related words, Kellenbach, Wijers, Hovius, Mulder, and Mulder (2002) examined differences in ERP components following the presentation of words from different grammatical classes and different semantic categories. In this study, Kellenbach et al. asked Dutch participants to attentively read nouns and verbs that belonged to one of three semantic categories: abstract (e.g., '*klemtoon', emphasis,* and *'bedroeven', grieve*); visual (e.g., '*nummerbord', license plate,* and *'dwarrelen', whirl*); and motor (e.g., *'pincet', tweezers* and *'afwassen', wash*). Kellenbach et al. analysed 50 ms sections of ERP waveforms that occurred following the presentations of these words looking for component differences elicited by different word types. The researchers report no interaction effects between grammatical class and word type, though they did observe that the earliest divergence for motor related words compared to abstract and visual words occurred at the 250 ms to 450 ms range. Although their ERP data did not indicate a specific cortical region involved in this process, Kellenbach et al. suggest that this is indicative that processing of semantic content of motor words occurs around this time-point. They go on to say that these data are evidence that "motor knowledge is accessed particularly quickly or efficiently, regardless of whether the stimulus is a manipulable object (noun) or an action (verb)" (p. 571). Kellenbach et al. propose that this rapid processing of motor-related words is due to the high salience of motor-word attributes (such as the cross-modal imageability of the object and associated action) compared to uni-modal visual words or abstract

words. Regardless of whether a word's 'motor salience' is truly the reason that motor-words are processed at this time point, this research still provides evidence suggesting that the 250 ms to 450 ms epoch likely encapsulates the earliest processing of content specific to motor words.

Like Kellenbach et al. (2002), other researchers have sought to investigate the time-course of processing motor related language and as per Pulvermüller et al. (2005), TMS has been used as a reliable method of altering motor cortex function in order to examine its role in action-language processing. Papeo, Vallesi, Isaja, and Rumiati (2009) examined the effect of TMS pulses delivered over the motor cortex at 120% of RMT at 170, 350, and 500 ms post stimulus onset. Papeo et al. examined changes in MEP amplitude as well as response accuracy and RT in trials where participants were either required to explicitly identify whether verbs were action-related or not (semantic task), or were required to decide on the number of syllables in the verbs (syllabic task). The verbs presented (in Italian) to the participants described either hand-actions (e.g., '*mescolo'*, *I stir*), non-hand actions (e.g., '*salto'*, *I jump*), or non-action (e.g., '*medito'*, *I wonder*). At all three latencies, accuracy was not significantly different among word types or between sham and TMS. Additionally, although no difference in RT was observed when the TMS pulse was delivered at 170 ms, a pulse at 350 ms led to slower RT compared to sham (control) to non-action words in the semantic task, and to both hand-action and non-hand action words in the syllabic task. When examining MEP results, Papeo et al. report that a TMS pulse delivered at 500 ms post-stimulus onset leads to increased MEP amplitude for hand action words when performing the semantic task compared to the syllabic task. As this effect is not seen elsewhere (at other latencies or for other word types), Papeo et al. suggest that the increased MEP amplitude at this time point in the

semantic task represents a facilitation in motor cortex activity when processing semantic information about hand-action words. Unfortunately, as the semantic and syllabic tasks are inherently different, RT data cannot be compared in order to detect if this increased facilitation impacts behavioural responses. That said, there was also no significant difference between RT in sham and TMS conditions for both tasks when the pulse was delivered at 500 ms, which indicates that this facilitation of the motor cortex during the semantic task is not linked to any significant TMS-induced RT change. Despite increased MEP amplitude at 500 ms, it is difficult to confidently conclude that this would be the ideal latency at which TMS should be applied to induce facilitation or inhibition, particularly without supporting behavioural results. Despite mixed results, as there were differences in the way TMS impacted the semantic versus the syllabic task, Papeo et al. were able to conclude that although there was evidence that the motor cortex is involved in processing semantic content of effector-specific action-related verbs. The author suggest that this process is not automatic upon perception of the word, and instead relies on some deliberate processing of semantic decision-making information in order to recruit motor networks.

Beyond lexical decision-making tasks, to gain a greater insight into the impacts of various single-pulse TMS parameters on behavioural response, it is also important to review the impact of TMS on simple RT. Pascual-Leone et al. (1992) conducted a study that examined the effects of single pulse TMS over the motor cortex with varied latency and intensity on RT in a simple go-signal task. Participants were asked to flex their right thumb or elbow as quickly as possible in response to a 'go' signal that was presented in either a visual, auditory, or tactile modality. RT was measured as the onset of EMG activity recorded from either the

bicep or abductor pollicis brevis (APB) muscle in the thumb. Pascual-Leone et al. administered a sub-threshold TMS pulse concurrently with the go-signal as well as intervals of 5 or 10 ms either side of the go-signal up to ±50ms. In that study, 'sub-threshold' intensity was below resting motor threshold (RMT) and was defined as the greatest intensity at which no MEPs were elicited that were larger than 50μV peak-to-peak. All results were compared against control condition, in which participants responded to the go signal without concurrent application of TMS. It was found that fastest reaction times could be seen at +5 to +10 ms post-stimulus onset, and compared to control trials, TMS at this latency improved RT by approximately 30 ms. The facilitative effect of TMS on RT is less apparent at latencies of -10 to -50 ms and +20 to +40 ms and was observed to actually inhibit/delay RT when TMS is delivered at +50ms. Pascual-Leone et al. also examined the impact of altering TMS intensity on RT by administering it at intervals of ±5% of sub-threshold intensity. RT was fastest (approx 25 ms faster than control) at sub-threshold intensity as well as 5% below. Again, this facilitative effect was much less discernible at -15% to -20% and +5% to +10% of sub-threshold intensity, and actually delayed RT at +15% to +25%.

It is difficult to directly compare the findings of Pascual-Leone et al. (1992) with Pulvermüller et al. (2005), as they used different methods to determine their TMS pulse intensity. As noted above, Pascual-Leone et al. tested using a sub-threshold intensity defined as the maximum intensity at which no MEPs were elicited, whereas Pulvermüller et al. found the minimum intensity at which MEPs were elicited (RMT) and then tested at 90% of that value. Despite the differences in methodology, it can comfortably be acknowledged that in both studies sub-threshold intensities promoted faster RT. Interestingly facilitation was seen in both the simple

go-signal task (Pascual-Leone et al., 1992) as well as in the vastly more complex linguistic decision making task (Pulvermüller et al., 2005). While it may be tempting to interpret this as an indication that a sub-threshold pulse to the motor cortex facilitates action regardless of complexity or modality of the task being attempted, it is important to note that Pascual-Leone et al. applied TMS to part of the motor-cortex responsible for the muscles used in the response (elbow flexion/thumb abduction), whereas Pulvermüller et al. asked participants to respond with a mouth movement while TMS was applied to arm and leg areas of the motor cortex. It is possible that the RT observed in the Pascual-Leone et al. study may be mediated by the direct impacts of the TMS pulse on the individual's ability to engage the motor cortex to produce the appropriate response, and this must be taken into account when examining limb RT while the motor cortex is being stimulated. If the Pascual-Leone et al. findings could be applied beyond facilitative effects of sub-threshold TMS, then it would also be expected that supra-threshold TMS would create inhibition to motor-cortex functioning and delay RT. As noted above, I sought to extend on the findings of Pulvermüller et al. to investigate the impact of a 120% TMS pulse on the motor cortex. Contrary to expectations, it was observed that a pulse of this intensity did not inhibit RT and therefore it could not be concluded that this had an impact on the processing of hand-related verbs in the motor cortex. As the processing of linguistic information is clearly more complicated than responding to a go-signal, it might also be suggested that when applying the Pascual-Leone et al. results to language research, the intricacies of linguistic processing need to be more broadly accommodated for. I suggested that future researchers may want to consider the potential facilitation or inhibition of motor cortex functioning at different latencies throughout the more extensive time-course of linguistic processing.

The current study aims to investigate the breadth of the impact of single pulse TMS by testing at both sub- and supra-threshold intensities at a range of latencies at which the motor cortex might be involved in language processing. As demonstrated by Skrandies (1992), Kellenbach, et al. (2002) and Papeo et al. (2009) there are a number of identifiable time-points during the processing of language where the brain's ability to differentiate motor information from other linguistic information may occur, and as demonstrated by Pulvermüller et al. (2005), the very specific protocol of a TMS pulse of 90% of RMT at 150ms over effector-specific areas of the motor cortex produces a facilitative effect on the processing of effector-specific verbs. Three early pulse latencies were selected for the current study, with the aim that these could impact potential motor cortex functioning during the early stages of semantic processing (from about 100 to 200 ms as identified in the Skrandies,1992 and Pulvermüller et al., 2005 studies). An obvious choice was the incorporation of the 150 ms latency as an early component as it has previously been used in both Pulvermüller et al. and Mertens'(2009) studies.

When deciding what other early latencies to include, it is important to consider the Pascual-Leone et al. (1992) findings as it could be argued that the 'golden' latency of 150 ms at which RT maximised in linguistic processing could be equated to Pascual-Leone et al.'s 0 to +5 ms optimal TMS latency. An extension of this would be the implication that RT facilitation would be less distinct, and perhaps even inhibited, the further the latency was moved (early or later) from 150 ms. Accordingly, the other early latencies chosen for TMS administration in this study were 120 ms and 180 ms.

Later components were more difficult to specify, as Pascual-Leone et al. (1992) only tested to +50 ms and the previous studies used methodologies too far

removed from that being employed in the current study. Papeo et al. (2009) (who assessed the impact of TMS pulses at later latencies) asked participants to semantically categorise the stimuli leading to average RTs approximating 560 ms to 1160 ms, whereas my previous methodology (Mertens, 2009), as well as that of Pulvermüller et al., relied on the automatic processing on linguistic information and elicited average RTs approximated at 400 to 460 ms and 540 ms to 590 ms respectively. With this in mind it was decided not to include the late 500 ms latency used by Papeo et al., as that TMS was likely to be triggered either during or after the participant's response, which obviously offers no insight into the linguistic processing that occurs prior to the response. This is not to say that processing isn't happening at this late stage, merely that the processing required for participants to respond in the current experiment (and perhaps in the Pulvermüller et al. study) would likely be completed prior to the 500 ms time-point. Indeed, as has been demonstrated in numerous studies looking at the N400 ERP component (Kutas & Federmeier, 2011), language processing (particularly integration of semantic information) is still occurring well into this later time-point. However, once again attempting to trigger TMS pulses at this late stage would likely occur around the time of response. As Papeo et al. had demonstrated that there were some semantically driven differences in RT (though not to action words) when TMS was applied at 350 ms, this was chosen as the latest time-point at which TMS would be applied in the current study. The other two 'late' latencies chosen for this study were 230 ms and 290 ms. While seemingly arbitrary, including these two latencies ensures reasonable coverage of the time between the latest early latency (180 ms) and 350 ms to ensure a more comprehensive picture of the impacts of TMS pulses throughout the different stages of language processing.

The rationale behind the choice of pulse intensities used in the current study is much less complex, and is largely based on including of pulse intensities used in previous studies (such as Pulvermüller et al., 2005; Mertens, 2009; and Papeo et al. 2009). With this in mind, 90% RMT (Pulvermüller et al., 2005) was chosen as the lowest pulse intensity, in addition to three higher intensities at 10% increments up to the upper limit of 120% RMT (Mertens, 2009; Papeo et al.2009).

Aims and Hypotheses

The current study aims to provide greater insight into the impact of delivering TMS at each of six latencies (120 ms, 150 ms, 180 ms, 230 ms, 290 ms, and 350 ms) and four intensities (90%, 100%, 110%, and 120% RMT) on the processing of hand-related verbs when applied over the motor cortex. It is anticipated that the results of Pulvermüller et al. will be replicated and a pulse intensity of 90% and latency of 150 ms will facilitate motor-cortex processing of hand-related verbs and result in faster RT compared to control (sham stimulation) and static (non-action) words.

Additionally, based on the lack of RT change in my earlier study (Mertens, 2009), it can also be hypothesised that a pulse of 120% at 150 ms will not significantly affect RT. While it is difficult to accurately predict the other specific interactions of pulse intensity and latency, based on the research conducted by Pascual-Leone et al. (1992) it can more broadly be hypothesised that as higher intensity pulses will result in slower RT than lower intensity pulses. Based on this same research as well as that of Pulvermüller et al. (2005), it can also be hypothesised that latencies away from the 'golden' 150 ms will result in slower RT compared to 150 ms, and latencies furthest from 150 ms (anything larger than +50 ms beyond 150 ms) will result in inhibition of motor cortex processing and actually delay RT compared to control (sham stimulation) and static words. Figures 6.1

(latency) and 6.2 (intensity) have been provided as an indication of hypothesised results. It should be noted that the change in RT shown in these figures is an arbitrary representation of change, and is intended to demonstrate increase or decrease in RT compared to control and not reflective of actual expected RT.

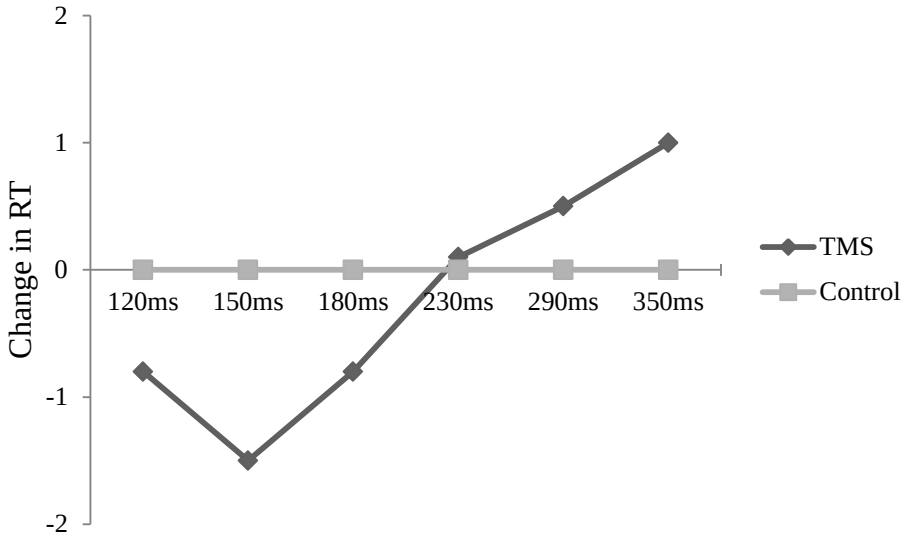

Figure 6.1. Estimation of hypothesised differences in RT between control and TMS groups for action words when pulse latency is manipulated. A latency of 150 ms is identified as pulse latency at which RT will be fastest. RT unit values are arbitrary and relative data-point positions are representative only.

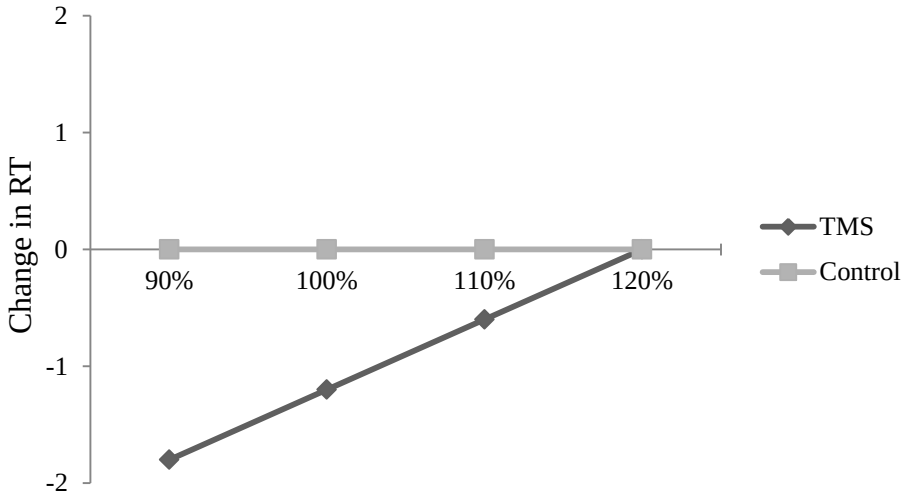

Figure 6.2. Estimation of hypothesised difference in RT between control and TMS groups for action words when pulse intensity is manipulated. An intensity of 90% will yield the fastest RT. RT unit values are arbitrary and relative data-point positions are representative only.

Regarding expected changes to MEP amplitude, it is anticipated that greater intensities will elicit larger MEPs, and latency should not significantly affect amplitude. As these are established direct physiological effects of TMS (Riehl, 2008), no research hypotheses will be made for this. Beyond these basic effects, based on the findings of Oliveri et al. (2004a), it may be expected that there will be specific difference in MEP amplitude between word types. Specifically, it is hypothesised that motor cortex excitability (as measured by MEP amplitude) will be greater for action words compared to static (non-action) words.

Method

Participants

Twenty-four individuals volunteered to participate in this research. Sixteen participants (five females) aged between 18 and 36 years (M = 20.56, SD = 4.50 years) were recruited as part of the experimental (TMS) group. The remaining eight participants (4 females) who were aged between 19 and 34 (M = 25.88, SD = 7.64 years) were recruited as controls (sham stimulation). Eighteen participants were first year university students who were recruited through the School of Psychology at the University of Tasmania and were awarded four hours course credit for their participation. The remaining seven participants were university students and staff not from first year psychology and they received no compensation for participating. This research was granted ethics approval by the *Human Research Ethics Committee (Tasmania) Network (no. H0009812).*

Participants self-reported that they were right-handed and had no language or visual deficits. Participants completed a consent form and medical questionnaire at the beginning of the first session (Appendices A and D) and no participants reported

contraindications to TMS (including; concussion, migraines, metal implants, pacemaker, or familial history of fits or seizures). All participants spoke English as their first language.

Apparatus

Word Lists

The list of real words and pseudo-words presented to participants at both sessions can be seen in Appendix E. This list of words was based on the stimuli used by Mertens (2009). The list of real words contained 40 common words that can be categorised as verbs. All 40 words can be classed as verbs according to Collins Concise Dictionary Plus, (Hanks, 1989). Of this list, 20 were determined to be verbs relating to or including hand movements (or "hand-action verbs" such as *draw, catch, cutting*) and 20 were determined to be verbs not related to any physical movement (or "static verbs" such as *dream, fear, choosing).* Additionally half of these real words were presented in their base verb form (e.g., *hope* or drop) and half were presented in their present participle and ended in –*ing* (e.g., *planning* or *cutting*). Present participle verbs were used to encourage participants to read the items displayed on screen as verbs. This was particularly important for verbs like *boxing*, in which the simple base form of the verb (*box*) could easily be read as a noun. Importantly, reading the words as verbs encouraged participants to interpret them as hand-actions. Items on both the hand-action and static lists were matched for length, syllabicity, and written frequency. Written frequency was determined using information from the MRC Psycholinguistic Database (Wilson, 1998), and as some on the present-participle verbs did not have frequencies listed (such as *believing*), written frequencies are associated base form of each word (i.e., *believe*).

Unfortunately, written frequency does not differentiate when the words are used in their verb or noun form, and is based on the occurrence of the word itself. The list of 40 pseudo-words contained pronounceable letter-strings that conformed to English language rules (such as *brope*, *telt*, *skrading*). These pseudo-words were matched with the real word list for length and syllabicity. Similar to the real words, half of the pseudo-words mimicked characteristics of past participle verbs (ended in *-ing*). The characteristics of the word lists are compared in Table 6.1.

Table 6.1.

Mean Characteristics of Stimulus Word-Lists Including K-F Written Frequency, (Standard Deviations in Parentheses).*

Word Sub-List	K-F Written Frequency	Syllables	String length (letters)
Hand-Action	70.63 (88.03)	1.6 (0.68)	5.85 (1.46)
Static	104.60 (75.83)	1.7 (0.66)	5.95 (1.82)
Psuedowords	-	1.6 (0.63)	5.95 (1.63)

*(Kucera & Francis, 1967, as cited in Wilson 1998)

Using a random number generator, all 80 words and pseudo-words were arranged into a random order to be presented to the participant during the experiment. Twenty-four versions of this randomly ordered 80-item word list were devised and a diagrammatic breakdown of the 80-item list can be seen in Figure 6.3.

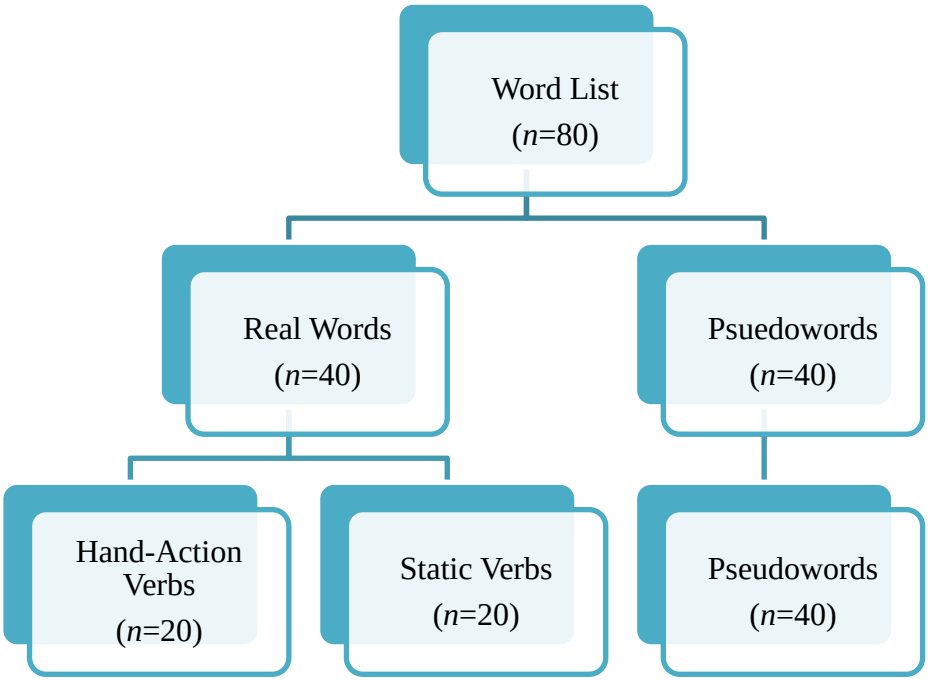

Figure 6.3. Grouping of word-types within the 80-item list.

Stimulus Presentation

Word lists were presented to participants via a computer screen. Words were presented one at a time using capital letters. White text was presented at the centre of the screen on a dark grey background and the words were 36 point Arial font. Each word was displayed on the screen for 100 ms. Each word was preceded and followed by a white cross in the centre of the screen on a dark grey background. There was a 2.5 s period between the presentation of each word. A TMS pulse was triggered at one of six predetermined time-points following the presentation of each item on the word list. This procedure is outlined in Figure 6.4 below.

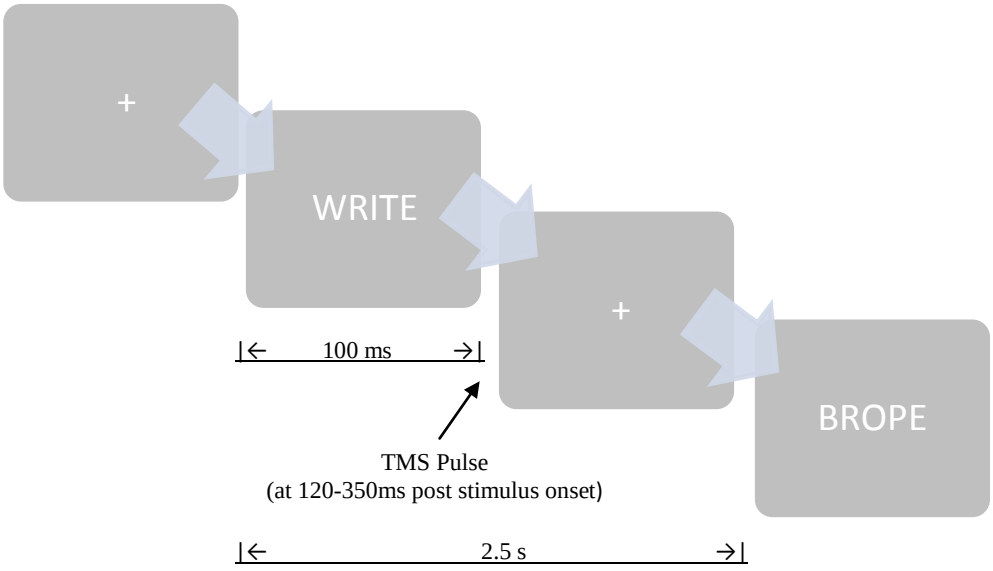

Figure 6.4. Sample of timing of word presentation and TMS pulse.TMS pulse timing varies depending on latency condition.

TMS and EMG Recording Apparatus

TMS was applied using a Magstim 200 (Magstim Co., Whitland UK) stimulator through a figure-of-eight coil (70 mm diameter of each wing). The sham TMS coil used in the sham condition did not deliver any electromagnetic pulse to the brain, however it did mimic the acoustic 'click' produced during the firing and was identical to the authentic TMS coil in both appearance and weight. EMG data were recorded from two sites – the first dorsal interosseous (FDI) muscle of the right hand, and the masseter muscle of the left side of the face. For FDI recordings, Ag/AgCl electrodes were attached at three sites –over the belly of the right hand FDI muscle, on the second metacarpophalangeal joint of the right index finger, and a reference electrode on the styloid process of the right radius. For masseter recordings, electrodes were attached at a further three sites – over the belly of masseter muscle in the left cheek and over the tendon of the masseter muscle below the left ear, and a reference electrode on the mastoid behind the left ear. EMG

activity was band-pass filtered (20 to 1000 Hz) and amplified using CED1902 amplifiers (Cambridge Electronics Design, Cambridge, UK) and then sampled using a CED Power1401 data acquisition system. Sweeps were collected 100 ms before to 1000 ms after the delivery of the pulse using Signal 3.08 software (Cambridge Electronics Design, Cambridge, UK).

TMS pulse intensity and latency were both manipulated independent variables in this study. TMS was delivered at four different pulse intensities – 90%, 100%, 110% and 120% of resting motor threshold. These cover a range stimulation intensities that incorporate those used in the protocols of by Pulvermüller et al. (2005) and Mertens (2009) with and reflect 10% intensity intervals between each one.

TMS latency was also manipulated and TMS was delivered at one of six time-points following the onset of the presentation of each item in the word-list (including pseudowords). The latencies for TMS pulses used in this study were 120 ms, 150 ms, 180 ms, 230 ms, 290 ms, and 350 ms following word presentation onset. This range of latencies was selected on the basis that they best reflected both early and late time-points at which the motor cortex could potentially be involved in linguistic processing and are based on the findings of Pulvermüller et al. (2005), Mertens (2009), Pascual-Leone et al.(1992), and Papeo et al. (2009).

Procedure

Participants attended two sessions within a laboratory at the University of Tasmania's Psychology Research Centre. At the beginning of the first session, participants were given a consent form, information sheet and a medical/history questionnaire to ensure their suitability for the study. The researcher gave

participants an opportunity to ask questions at the beginning of and throughout the sessions. Participants were requested to wash their right hand and wrist with soapy water in order to remove excess dirt from the skin. An abrasive gel was then used to abrade the area of skin over electrode sites. Alcohol swabs were then used to wipe away dead skin cells from these areas. Alcohol swabs were also used to remove makeup and debris from the area of the left cheek over the masseter muscle and mastoid. Participants were requested to ensure their faces were clean shaven prior to attending the first session, however disposable razors were available to remove facial hair when necessary.

Electrodes were then attached at specific sites over the FDI and masseter muscles. Participants were seated in a comfortable adjustable chair approximately 60cm from the computer monitor. The electrodes were then connected the amplifier and recording computer, and their outputs were assessed to ensure they were accurately recording muscle activity. Electrodes were adjusted if necessary to ensure conductivity, and participants were requested to relax their muscles if involuntary motor activity was visible in the recordings. Participants were asked to practice their 'jaw response' by holding their mouth closed and clenching the muscles at the back of their jaw.

The ideal location for application of TMS (or the "hotspot") was determined by holding the TMS coil over the left hemisphere's motor cortex (approximated as 5cm lateral and 1cm anterior of the midpoint of the head). The coil then repeatedly discharged as the position and orientation of the coil was adjusted to find the cortical location that provided the maximal MEP amplitude in the right FDI. Once the hotspot had been determined, an ink marker was used on the scalp of the participant to mark the position of the coil. Maintaining the coil over the hotspot, the researcher

then determined resting motor threshold (RMT, defined as the minimum charge intensity needed to evoke at least 3 MEPs ≥ 50μV on 5 consecutive trials). Using the computer presentation program, participants then practised the task before beginning the test trials. The practice task consisted of a short 15-item list which contained no experimental target words.

In each test trial, participants viewed the list of 80 items. They were informed they were going to view a list of verbs that were either commonly used real words, or made-up words. Participants were instructed to only respond with their jaw when they saw a word that they recognised as a real English word. Participants were encouraged to respond as quickly and as accurately as possible. During each list, the TMS coil was positioned over the hotspot and a pulse was triggered following the presentation of each item on the list. For participants in the control condition, a sham TMS procedure was implemented. In the sham procedure, participants also viewed the lists of 80 items, however a sham coil was used to mimic the acoustic properties of authentic TMS procedure, without delivering any actual stimulation. Threshold and hotspot determination was still conducted authentically with control participants, however before the testing began, participants were informed that the experimenter needed to change coils, and that although this coil operates similarly to the other coil, they may not feel any physical sensation from the second coil. The sham coil was then placed over the hotspot, and was triggered at the presentation of each item on the list.

Participants completed 12 test trials in each session, viewing the entire list of 80 items in a different predetermined randomised order in each trial. During each trial participants would receive TMS (experimental group) or sham-TMS (control group) following the presentation of the item at one of the four levels of intensity

and at one of the six latencies. The order of the trials was pseudo-randomised within blocks which contained four trials each and the order of the blocks was counterbalanced across participants and across sessions. Therefore, over two sessions participants completed six blocks containing four trials each (totalling 24 trials). Over these two sessions participants received TMS (or sham-TMS) for each level of intensity and latency. Participants were contacted to arrange a second session at the closest convenience within 7 - 14 days after their first session (*M* = 10.00, *SD* = 5.75 days). Hotspot and RMT were also determined independently for each session. Fluctuations in RMT between sessions were within acceptable limits (*M* = 1.40, *SD* = 1.47 percent of maximum stimulator output).

Design

This research employed a 2 x 2 x 4 x 6 mixed measures design with TMS (TMS, Sham) as the between subjects factor and Word Type [Action, Static], Intensity [90%, 100%, 110%, 120%], and Latency [120 ms, 150 ms, 180 ms, 230 ms, 290 ms, 350 ms] as the within subjects factors. The dependent variables were RT in response to target words, and MEP amplitude during the TMS condition.

Data Analysis

Initial examination of RT data indicated that in many of the trials in which TMS was delivered at a latency of 350ms, participants' responses were initiated prior to the delivery of the TMS pulse. Clearly, a TMS pulse occurring after a response could not possibly influence the neural pathways involved in the processes leading up to the generation of that response. It was therefore decided that all 350 ms latency trials would be omitted from further analyses. Furthermore, a number of responses

were observed to have initiated prior to administration of TMS in the 230 ms and 290 ms conditions, as reasoned above, these individual RT data (a total of 10.57% total responses from these two conditions) were omitted from analyses.

Similar to Pulvermüller et al. (2005) RT was taken as 25% of the maximal amplitude of the EMG burst recorded from masseter muscle activity following the presentation of each word. Based on methodology employed by Garry and Franks (2000), EMG trace data were rectified and a low-pass filtered version (Butterworth, 50 Hz cutoff) of this signal was then superimposed over this trace using Signal 3.08 (Cambridge Electronics Design, Cambridge, UK). Activity onsets were tagged using this software and individually checked by the researcher who was blind to word condition before being exported to a statistical analysis software package. A mixed Analysis of Variance (ANOVA) was conducted to investigate potential differences in RT between the TMS and control group for Action or Static words across each level of pulse Intensity and Latency. Hedge's *g* was employed as a measure of effect size.

As MEPs are only produced during active TMS administration, MEP data were analysed for all words in the TMS participant group only. Using the Signal 3.08 software program (Cambridge Electronics Design, Cambridge, UK), MEP amplitudes were derived by measuring the difference in μv between the maximal and minimal peaks of the waveform.

MEP amplitudes were then exported to a statistical analysis software package and, similar to the RT data manipulations, outliers (MEPs that exceeded 2.5 standard deviations of the mean MEP amplitude) were removed from the raw data. MEP amplitudes were log transformed in order to improve the approximation to normality (Wasserman, 2002). Using the log transformed values, a repeated measures ANOVA

was performed to compare MEP amplitude for across all levels of Word Type, Latency, and Intensity.

Results

Behavioural Data

Following a boxplot analysis, it was determined that two participants in the TMS condition and one participant in the control group recorded average RTs beyond 1.5 standard deviations of the mean for many conditions within their group. Data for these participants were removed to minimise positive skew of the data. Mean RT data averaged across participants within each group for all pulse manipulations are presented below for Static words (Table 6.2) and Action words (Table 6.3) with the omission of these participants.

Table 6.2.

Mean RT (Shown in ms) for TMS and Control Groups for Static Words for Latency and Intensity Conditions (Standard Deviations in Parentheses).

	Pulse Intensity							
	90 %		100 %		110 %		120 %	
Latency	TMS	Sham	TMS	Sham	TMS	Sham	TMS	Sham
120 ms	402.18 (35.13)	432.55 (56.78)	397.22 (46.75)	441.27 (62.03)	385.19 (62.74)	419.98 (46.70)	381.38 (54.87)	440.21 (89.90)
150 ms	374.29 (54.04)	434.44 (40.93)	407.97 (61.14)	418.08 (49.97)	388.16 (55.10)	426.60 (34.11)	388.66 (62.84)	415.53 (58.25)
180 ms	392.13 (55.08)	419.99 (41.34)	386.74 (48.93)	409.52 (48.65)	386.10 (62.69)	413.76 (47.84)	388.89 (59.14)	415.86 (39.42)
230 ms	389.44 (36.28)	405.55 (53.14)	385.23 (46.24)	431.25 (45.79)	392.80 (59.97)	416.61 (49.13)	401.33 (55.18)	419.02 (48.82)
290 ms	407.21 (29.04)	439.86 (47.40)	414.76 (37.23)	453.75 (62.93)	410.08 (34.98)	430.70 (59.49)	414.44 (42.01)	419.09 (38.87)

Table 6.3.

Mean RT (Shown in ms) for TMS and Control Groups for Action Words for Latency and Intensity Conditions (Standard Deviations in Parentheses).

	Pulse Intensity							
	90 %		100 %		110 %		120 %	
Latency	TMS	Sham	TMS	Sham	TMS	Sham	TMS	Sham
120 ms	402.97 (34.66)	488.49 (54.59)	404.69 (41.61)	450.96 (56.04)	391.40 (56.62)	428.21 (49.35)	395.01 (59.46)	458.13 (102.94)
150 ms	392.00 (66.79)	454.11 (56.39)	410.34 (61.32)	412.73 (31.46)	385.14 (51.07)	433.99 (44.15)	391.75 (64.16)	406.61 (55.23)
180 ms	379.55 (57.46)	420.93 (39.82)	386.31 (45.41)	411.18 (55.54)	387.68 (54.11)	424.52 (45.26)	387.55 (57.72)	432.16 (30.14)
230 ms	393.47 (46.94)	409.77 (32.98)	389.47 (51.06)	446.86 (40.98)	398.41 (56.62)	425.65 (52.68)	394.32 (53.47)	423.63 (50.52)
290 ms	412.03 (32.61)	448.22 (42.61)	420.05 (31.80)	446.46 (54.39)	425.56 (37.00)	445.29 (57.80)	414.42 (38.75)	423.42 (37.11)

Mean RT data for 22 participants were analysed using a 2 (TMS) x 2 (Word Type) x 4 (Intensity) x 5 (Latency) mixed ANOVA. It was found that there was a trend towards a main effect of TMS condition, $F(1,20) = 3.64$, $p = .071$, $g = 0.85$, indicating a large effect approaching significance with the means showing that regardless of word type, pulse latency or intensity, participants in the TMS group ($M = 396.28$, 95% CI [374.81, 417.76]) produced faster RTs than control participants ($M = 428.87$, 95% CI [400.46, 457.29]) . There was also a significant main effect of word type, $F(1,20) = 9.46$, $p = 0.006$, $g = 0.14$, and the means indicate overall RT was faster to Static words ($M = 409.82$, 95% CI [391.67, 427.98]) than Action words ($M = 415.34$, 95% CI [397.68, 432.99]). Although this difference is statistically significant, it is very small (less than 6 ms). There was no statistically significant main effect of Intensity, $F(3,48) = 0.60$, $p = .58$, Huynh-Feldt corrected, however there was a significant main effect of Latency, $F(3,58) = 5.84$, $p = .002$, Huynh-Feldt

corrected. A quadratic contrast was also observed in the Latency data, $F(1,20) = 13.27$, $p = .002$. As is shown in Figure 6.5, the quadratic contrast for latency indicates a pattern in the data demonstrating that fastest RT was seen when TMS pulse (both real and sham) was triggered at a latency of 180 ms and that RT was slower when pulse was administered prior to or after the 180 ms time-point. For comparison, figure 6.6 shows RT for TMS and Sham groups on different lines. It can be seen that the shape of the contrast observed in the collapsed data is reflected in the individual data for both the TMS and Sham groups.

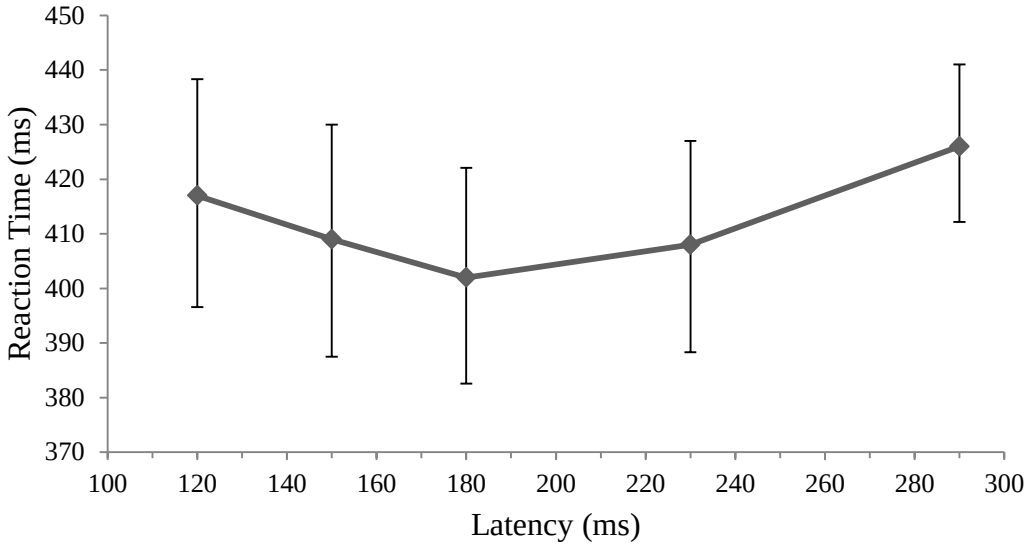

Figure 6.5. Mean RT for Latency Conditions Demonstrating Quadratic Contrast with Data Collapsed Across TMS, Word Type and Intensity. This trend shows a pulse latency of 180 ms elicits the shortest RT with latencies either side of this eliciting longer RT. Error bars represent 95% confidence intervals.

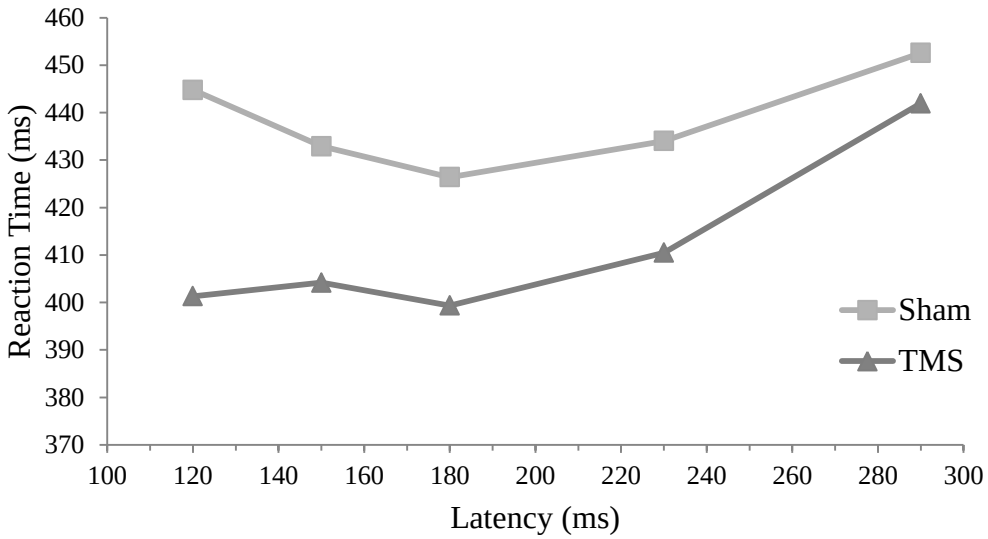

Figure 6.6. Mean RT for Latency Conditions for both Sham and TMS Groups. Data Collapsed Across Word Type and Intensity. This interaction was not significant, however this is included to demonstrate RT patterns that are largely similar when data is separated by TMS/Sham group.

The ANOVA also produced a significant three-way interaction between Word Type, Latency, and Intensity conditions, $F(12, 234) = 2.18$, $p = .014$, Huynh-Feldt corrected. Sidak corrected pairwise comparisons were conducted to examine the data for potential differences driving the significant interaction. It was found that a pulse delivered at 90% intensity at 150 ms led to faster RT for Static words than for Action words, regardless of whether participants were in the TMS or control group ($p = .005$, $g = 0.32$). RT to Static words was also significantly faster regardless of TMS condition when a pulse was triggered at 120 ms at 120 %, $p = .040$, $g = 0.20$, 180 ms at 120 % $p = .029$, $g = 0.20$, and at 290 ms at 110%, $p = .001$, $g = 0.31$.

There was no significant interaction between Word Type and TMS, $F(1,20) = 1.09$, $p = .31$, Word Type and Latency, $F(4,72) = 1.09$, $p = .37$, Huynh-Feldt corrected, Word Type, Latency, and TMS, $F(4,72) = 0.93$, $p = .44$, Huynh-Feldt corrected, Word Type and Intensity, $F(2,45) = 0.61$, $p = .57$, Huynh-Feldt corrected ,

Word Type, Intensity, and TMS, $F(2,45) = 0.48$, $p = .17$, Huynh-Feldt corrected, Latency and Intensity, $F(7,141) = 0.76$, $p = .64$, Huynh-Feldt corrected, Latency, Intensity and TMS, $F(7,141) = 1.48$, $p = .17$, Huynh-Feldt corrected, or four way-interaction between Wordtype, Latency, Intensity, and TMS, $F(11,225) = 0.52$, $p = .90$, Huynh-Feldt corrected.

To provide a closer comparison to the results of Pulvermüller et al. (2005), it is important to analyse the data using comparable methodology. Pulvermüller et al. evaluated RT to Arm words compared to Leg words and although participants responded to distracter words as well, RT data to distracter words were not reported. In the current study, the static words could be considered distracters, and therefore it was decided that a further analyses would be conducted examining RT to action words only. Again, the Mean RT data for 22 participants (grand means for action words shown in Table 6.2) were analysed using a 2 (TMS) x 4 (Intensity) x 5 (Latency) mixed ANOVA. It was found that there was trend towards a main effect of TMS condition, $F(1,20) = 4.14$, $p = .055$, $g = 0.90$, again indicating a large effect approaching significance with the means showing that regardless of pulse latency or intensity, participants in the TMS group (M =398.11, 95% CI [376.81, 419.40]) produced faster RTs than control participants (M = 432.57, 95% CI [404.40, 460.73]). There was also a main effect of pulse Latency, $F(3,57) = 5.76$, $p = .002$, Huynh-Feldt corrected. Similar to the previous analysis, Sidak-corrected pairwise comparisons revealed that RT in the 290 ms condition was significantly delayed compared to the 150 ms ($p = .029$, $g = 0.44$), 180 ms ($p < .001$, $g = 0.68$), and the 230 ms conditions ($p = .024$, $g = 0.50$). The quadratic contrast of Latency demonstrated earlier also persisted in the Action words only RT data, $F(1,20) =$

14.51, p = .001, overall demonstrating that the results are reasonably stable regardless of the inclusion/exclusion of RT to Static words.

Electrophysiological Data

Following the removal of participants with outlying RT data, MEP data were log transformed for the remaining 14 participants in the real-TMS condition, and the average MEP amplitudes for all conditions can be seen in Table 6.4.

Table 6.4.

Log Transformed Mean MEP Amplitudes for Each Level of Word Type, Latency, and Intensity Conditions (Standard Deviations in Parentheses).

	Pulse Intensity							
	90 %		100 %		110 %		120 %	
Latency	Static	Action	Static	Action	Static	Action	Static	Action
120 ms	-4.03	-3.95	-2.02	-2.18	-.65	-.73	.10	.15
	(.41)	(.54)	(1.04)	(1.07)	(.84)	(.79)	(.54)	(.60)
150 ms	-3.92	-3.97	-2.09	-2.06	-.88	-.93	.02	.06
	(.63)	(.69)	(.85)	(.98)	(.63)	(.80)	(.71)	(.68)
180 ms	-4.14	-4.13	-2.66	-2.71	-.72	-.78	.07	.08
	(.53)	(.52)	(.70)	(.80)	(.97)	(.86)	(.64)	(.64)
230 ms	-3.91	-4.02	-2.16	-2.28	-.68	-.62	.28	.26
	(.56)	(.52)	(.87)	(.92)	(.77)	(.87)	(.74)	(.77)
290 ms	-3.69	-3.83	-1.73	-1.98	-.83	-.82	.05	.02
	(.85)	(.73)	(.88)	(.81)	(.67)	(.66)	(.75)	(.81)

Mean MEP amplitude was analysed using a 2(Word Type) x 4(Intensity) x5 (Latency) repeated measures ANOVA. A significant main effect of Word Type was found, $F(1, 13) = 12.35$, p = .004, g = .10. Despite means showing Static words (M = -1.68, 95% CI [-1.92, -1.44]) yielding larger MEP amplitudes than Action words (M = -1.72, 95% CI [-1.96, -1.48]), the effect size indicates that this difference is negligible. There was also a significant main effect of Intensity, $F(3, 35) = 269.67$, p < .001. Sidak-adjusted pairwise comparisons revealed all MEP amplitudes differed significantly between all TMS intensities, with 90% yielding the lowest MEP amplitude (M = -3.96, 95% CI [-4.17, -3.75]), followed by 100% (M = -2.19, 95% CI

[-2.54, -1.83]), 110% (*M* = -0.77, 95% CI [-1.11, -0.43]), and 120% with largest MEP amplitude (*M* = 0.11, 95% CI [-0.21, 0.43]). Effect sizes ranging between *g* = 1.53 and *g* = 8.59 for comparisons of these means illustrate that this is a strong effect as would be expected with the increase of pulse intensity. There was also a trend toward a significant interaction between Word Type and Intensity, $F(3, 39) = 2.67$, $p = .06$. Sidak-adjusted pairwise comparisons revealed no significant difference in MEP amplitude between word types at 90%, 110%, and 120% intensity levels. At 100% intensity, Static words (*M* = -2.13, 95% CI [-2.48, -1.79]) yielded higher MEP amplitude than Action words (*M* = -2.24, 95% CI [-2.61, -1.87]), though this difference was negligible, *g* = .18. There was no significant Latency main effect, $F(4, 44) = 1.32$, $p = .28$, nor a significant interaction of Word Type by Latency, $F(3, 37) = 0.71$, $p = .54$, Latency by Intensity, $F(12, 156) = 1.58$, $p = .10$, nor a three-way interaction, $F(9, 116) = 0.76$, $p = .66$, all following Huynh-Feldt corrections.

Discussion

The aim of this study was to determine how a TMS pulse delivered over the hand area of the motor cortex would impact RT when processing motor-related words. Based on the findings of previous authors (Mertens, 2009; Papeo et al., 2009; Pascual-Leone et al., 1992; Pulvermüller et al., 2005,), TMS pulse intensity and latency were varied in order to examine the limits of potential effects reported in the literature.

Before examining the behavioural results, a brief discussion of the MEP results is necessary. It was expected that MEP amplitude would be higher for action words compared to static words as a reflection of increased motor cortex excitability when viewing these words (as reported by Oliveri et al., 2004a). The difference in

MEP amplitudes between action and static words was negligible, and if anything was trending toward the opposite direction of that expected; therefore this hypothesis was not supported. The differences between these findings and those of Oliveri et al. may be due to the different nature of the task presented to participants This may be due to the different stimulus design (Oliveri et al. showed participants verbs and *nouns*), or may be a result of Oliveri et al. using paired-pulse TMS to measure excitability, compared to the single-pulse methodology used in the current study.

Regarding the behavioural results, the broad initial hypotheses were based on the notion that the 'golden' intensity and latency combination (90 ms at 150%) reported by Pulvermüller et al. (2005) would elicit faster RT to action words compared to static words. Further to this, based on the findings of Pascual-Leone et al. (1992) it was broadly hypothesised that higher intensity pulses would result in slower RT than lower intensity pulses and that latencies away from the 'golden' 150 ms would result in slower RT compared to 150 ms, with latencies furthest from 150 ms delaying RT compared to static words.

The large effect size and trend towards significance for the main effect of TMS group in the analyses indicate that overall the TMS group had a faster RT in this task, however, there was no indication in the results that this reduction in RT is specific to action words compared to static words. Additionally, although it was found there was statistical difference in RT between action and static words, the actual difference (approximately 6 ms) is small enough to be dismissed.

Contrary to the anticipated speeding of RT to action words when the a pulse of 90 % RMT was delivered at 150 ms post stimulus onset (as per Pulvermüller et al., 2005), it was found that RT was significantly faster to static words compared to

Action words at this Intensity/Latency condition. While this result is surprising, considering that Pulvermüller et al. did not report the RT to distracter (control words) in their study, it is possible that significant results they observed were due to the delayed RT to leg-action words compared to arm-action words when stimulation the hand area of the motor cortex (and vice versa for in arm-words when the leg-area of the cortex was stimulated). Furthermore, it was found that this effect of faster RT to static words was found at a number of other Intensity/Latency combinations, specifically 110% with 230 ms, 120% with 120 ms, and 120% with 180 ms. It should be noted that while all four of these comparisons were significant, the effect sizes were small to moderate (g = 0.20 to 0.40) and actual differences in RT only ranged between approximately 10 and 19 ms.

Regarding the expected general Latency and Intensity effects (specifically the slowing of RT as pulse intensity increases and the speeding of RT when pulses were delivered at or around 150 ms), it was found that there was a significant main effect of Latency only. Importantly, the lack of significant interaction with TMS implies that any main effect of Latency or Intensity cannot be interpreted as a direct result of transcranial magnetic stimulation of cortical networks, and therefore a more detailed examination of these findings in warranted.

TMS and Sham protocols were identical, save the delivery of cortical stimulation. In the Control group (using the sham protocol), participants still felt the tapping sensation of the coil against their scalp, and the audible click discharged from the coil also occurred in both sham and TMS protocols. As is shown previously in Figure 6.5, RTs were fastest when the pulse was delivered at 180 ms, and slower at when TMS was delivered prior to or after 180 ms. The lack of interaction between TMS condition and pulse Latency indicates that this observation is true for both

TMS and Control participants, and therefore, latency effects cannot be attributed to the physiological effects of TMS, but may actually be the result of the sensory cues provided by a discharging TMS coil.

Previous researchers have reported RT facilitation resulting from the acoustic cueing of a TMS coil. In a task that required participants to manually respond to a visual go-cue, Marzi et al. (1998) compared RT between a standard TMS condition, an acoustic-only condition in which a stimulation-free acoustic 'click' cue was employed, and a control condition with no TMS or acoustic cue. They reported that RT was faster in both the TMS and acoustic-only conditions, compared to control. However as there was no difference between the TMS and acoustic-only conditions, the researchers concluded that the speeded RT seen in the TMS condition was likely due to the acoustic stimulation. It should be noted that the researchers also found that there was a delay in RT in the TMS condition compared to the acoustic only condition (but not to control) when the visual stimulus was presented in the visual field contralateral to the responding hand, however this has no bearing on the current study. Similar results were also reported by Terao et al. (1997) who found that in a simple visual RT task, participants showed faster RT (by approximately 20-50 ms) when sub-threshold TMS was applied over the motor cortex, as well as when it was applied over other unrelated cortical areas (central and parietal midline). Importantly, they also found similar RT facilitation when a sham TMS procedure was administered, and they concluded that most of their findings were driven by the cueing effects of the acoustic 'click' that accompanies TMS. Furthermore, the initial hypotheses of the current study were based on observations from RT in a simple go-task reported by Pascual-Leone et al. (1992), who found a U-shaped function not dissimilar to that of the current study. However, Pasucal-Leone et al. (1992) used no

sham TMS procedure, and their control protocol was merely RT to a go-signal sans TMS. Therefore, it could be hypothesised that fluctuations in RT due to changes in latency reported in this previous study were at least partially due to the acoustic cueing effects of the discharge the TMS coil.

Regarding the findings in the current study, ultimately, while RT in the TMS participants was overall still faster than RT in Control participants, it is reasonable to expect that changes in RT across latencies mirrored in both TMS and Control conditions is a result of the cueing effect of the TMS-click (see Figures 6.5 and 6.6). Despite the acoustic cueing effects, the faster RT demonstrated by TMS participants deserves further exploration. One possible explanation is that as TMS and Sham protocol were administered on different participants the differences in RT observed is merely representative of group differences unrelated to the presence/absence of TMS. However, as group allocation was random, it is improbable that one group would be faster or slower than the other based on chance.

In the current study, TMS participants responded faster than Control participants when RT for Action and Static words were combined (approximately 31 ms faster) and to Action word RT alone (approximately 34 ms faster). Group differences aside, perhaps motor-cortex involvement in language processing regardless of word-type explains faster RT in TMS participants. Meister et al. (2003) used TMS to examine the excitability of the hand and leg areas of the motor cortex while participants either read aloud or produced non-verbal mouth movements. They found that during reading, motor cortex excitability was increased in the hand-area of the motor cortex in the dominant hemisphere compared to before or after reading. This effect was not observed in the contralateral hemisphere, or in the leg area of the motor cortex. During non-verbal mouth movements, they observed slightly increased

motor cortex excitability bilaterally. Although this study does not examine changes in RT, as noted by Meister et al., the observation of excitability changes in the dominant hand motor cortex during reading aloud supports the notion of a functional connection between the hand motor area and language processing. Despite this finding, many other researchers have found that motor cortex excitability is not modulated by all words, but is only impacted by the processing of action words (Oliveri et al., 2004a). This specifically occurs when action words are presented visually (Labruna, Fernandez-del-Olmo, Landau, Duque, & Ivry, 2011) and when action words are presented in sentences (Tettamanti et al., 2005).

While Meister et al. (2003) did not examine RT in their study, nor did they have a measure of RT, their findings do still have some bearing on the interpretation of the results of the current study. Tomasino, Fink, Sparing, Dafotakis, and Weiss (2008) suggest that the reading component of language processing is not key to motor cortex involvement, but it is in fact the motor-imagery that can accompanies the processing of language that engages motor cortical networks. In that study, participants either read silently, judged whether a word described a rotating hand movement (imagery condition), or judged the word's written usage frequency, and were required to respond on the completion of each task. The researchers then compared RT when TMS was applied over the motor cortex or the central vertex (control). These researchers also varied TMS pulse latency (at 150 ms, 300 ms, 450, 600 ms and 750 ms post stimulus onset) but found no effect of pulse latency in any condition. This could be interpreted as evidence that a latency effect does not always appear, or that it does not appear at the late-processing time points chosen by Tomasino et al. Despite the finding that latency manipulation had no impact on RT, Tomasino et al. report that RT was facilitated when TMS was applied, but only in

the imagery condition. The reading silently and frequency judgement conditions did not yield any significant differences in RT. Tomasino et al. concluded that the motor cortex is involved in so far as motor-imagery is concerned and is not observably engaged when judgements about the word's frequency are made. Imagery was not measured in the current study, and while it is impossible to tell if participants automatically evoked mental imagery of the actions they were reading, they were instructed to perform an imagery-free word-judgement task (real-word validity). The observation that when TMS was applied RT was faster in a judgement task indicates that imagery may not be the key to motor cortex involvement.

Ultimately the results of the improved RT in the TMS condition to both hand-action and non-action words are contrary to notion that underpins much of the research in this field — that action-words specifically trigger motor cortex engagement in language processing (Labruna et al. 2011; Oliveri et al.2004a). In light of the large effect size (g = 0.85) the observed TMS versus Control RT trend is still important to acknowledge. While the actual reduction in RT of approximately 31-34 ms seen in the TMS group could be dismissed as relatively small, it is within the range of RT differences reported by previous researchers (e.g., the visual RT task by Terao et al., 1997). Additionally, it was observed that there was a lack of support for the ideal facilitative latency/intensity combination of 150 ms/90% (Pulvermüller et al., 2005) and based on the quadratic contrast, it could be inferred that 180 ms may be the ideal latency at which the pulse facilitates RT. However, as this effect was generally reflected in both TMS and Control participants, it could be suggested that 180 ms is the ideal moment for an acoustic cueing trigger to facilitate RT. These results do not therefore indicate a specific latency at which the *physiological* effects of TMS on the motor-cortex facilitate RT but merely that the TMS group had faster

RT overall, with both groups' data reflecting the quadratic contrast that indicated a coil discharge (real or sham) at 180 ms elicits the shortest RT.

Regarding the impacts of TMS on the role of the motor cortex in language processing these results may be taken to imply that TMS speeds language processing regardless of the word type. Alternatively, it may be argued that TMS is having an impact on other aspects of this task, such as word-validity judgement or the physical jaw-clench response. That said, there is no empirical reason to believe that the motor cortex would be involved in a word-validity decision, and the cortical region stimulated in this study was very specifically the hand-area, and would have been very unlikely to recruit motor networks responsible for a jaw-clench response. While these possibilities cannot be emphatically dismissed, they are highly unlikely and therefore rejected as possible explanations for these results. Ultimately these results contrast with that of previous research (Labruna et al., 2007; Oliveri et al.2004a; Pulvermüller et al., 2005) and imply that application of TMS over the motor cortex facilitates RT in this lexical-decision making task, regardless of the word type.

Results from experiments like this could be strengthened in future studies using repeated measures methodology, ensuring the same participants complete both the TMS and sham conditions. This would ensure some certainty that the results could not be attributed to differences between the groups. Due to the unexpected acoustic cueing effect observed in the Control (sham) data, these data are not a true indication of how these participants respond if no 'click' cue or stimulation was provided. Comparisons in this study are therefore an indication of the difference in RT between an acoustic cue *with* stimulation, and an acoustic cue *without* stimulation. It would be beneficial for future researchers to include a 'click-and-stimulation-free' condition in subsequent experiments. That said, the sham procedure

is highly important as it allows researchers to avoid the pitfalls of assuming that any effects seen in a real-TMS procedure are attributable entirely to stimulation. With the inclusion of repeated measures and other control conditions, future researchers would also therefore likely need to reduce the number of latency and intensity conditions, or reduce the number of words presented to participants, to stave off fatigue effects that would likely arise out of such a time-intensive procedure.

In summary, the aim of this study was to provide more insight into the role of the motor cortex in processing action-related language, with a specific focus on words related to hand actions. Contrary to expectations, participants in the TMS condition responded faster than Control participants to all words, regardless of whether or not they were action-words. It was also expected that a specific pulse latency and intensity combination (150 ms and 90%) would elicit the fastest RT. However, it was observed that there were no overall intensity effects and that 180 ms may be the pulse latency which elicits the fastest RT. Furthermore, this effect was reflected in data from both TMS and Sham groups indicating that while the TMS group had an overall faster average RT, the cueing effect of the coil 'click' may be the cause of specific speeding at the 180 ms latency. Overall these results do indicate motor cortex involvement in this lexical decision making task, and that TMS of the motor cortex can facilitate RT. Importantly, this effect was observed to be non-specific as it applied to both hand-action words and non-action words.

Chapter 7: Study Two

Introduction

The mirror neuron system - the proposed cortical network responsible for the processing of meaningful actions - is thought to encompass a number of specific cortical areas, including the motor and pre-motor cortices (Grafton et al.,1996; Rizzolatti et al., 1996). If the motor cortical areas are involved in the processing of meaningful actions, then it would logically follow that the meaningful hand movements that create gestures could potentially activate these sections of the mirror neuron network. To investigate this notion further, Willems, Özyürek, and Hagoort (2007) conducted an fMRI study examining cortical activity of participants observing speech streams accompanied by gesture. In that study, 16 participants viewed a recording of an actor with their face occluded who spoke a sentence while producing one iconic gesture for a target word that was either congruent or incongruent with the rest of the utterance (e.g., "He should not forget the items that he *wrote/hit* on the shopping list."). In addition to this the gesture produced either matched or mismatched the target word, creating an extra congruency variable. Therefore stimuli contained target words that were either congruent or incongruent with the speech stream and were accompanied by a gesture that either matched the target word, sentence, or neither. Ultimately, Willem's et al. recorded fMRI data under four conditions: gesture and target word both matching the sentence, target word mismatching, gesture mismatching, or gesture and target word both mismatching the sentence (however matching each other). Using the 'correct' (complete matching) condition used as a control, Willems et al. were able to detect

changes in cortical activity specific to the processing of mismatching speech and gestural information.

Results from this fMRI study showed increased activation in the left inferior frontal cortex (or Broca's area and surrounding cortex) in all three of the mismatching conditions (Willems et al. 2007). Willems et al. suggest that the involvement of the left inferior frontal cortex supports the notion that this area serves as a general "unification site" for linguistic comprehension. Taking into account the underlying linguistic nature of gestures (e.g., McNeill, 1985; Rossini, 2012), it logically follows that this area would be recruited for this task. Willems et al. account for the increased activation during the mismatching task being the result of the greater difficulty of integrating incongruent information. Additionally, for the target gesture mismatch condition, increased cortical activation was observed bilaterally in the premotor cortices. The authors interpreted this as evidence of increased cognitive load on the motor cortex when processing the mismatching gestures. In light of previous research supporting the existence of the mirror neuron system (Grafton et al.,1996; Rizzolatti et al., 1996), it could be argued that processing a meaningful action (in this case, gesture) would create activity in cortical motor areas regardless of whether it matches with speech. Willems et al. suggest that the observation that increased activity in pre-motor cortices indicates that speech has the potential to impact the processing of gestural information, increasing cognitive load when gesture and speech are not congruent.

As in the above study, some of the few published studies on investigating the neural underpinning of gesture processing are guided by mirror neuron theory, and therefore the cortical areas examined often include Broca's area, as well as the motor cortex. In another study examining the potential links between mirror neuron

systems and gestural processing, Skipper et al. (2007) investigated whether the semantic quality of a gesture was reflected in the neural networks it activated. In that study, fMRI data were recorded for 12 participants while they watched a recording of an actor telling a short story. The story was presented under four different conditions: *Gesture,* in which the actor produced gestures that related to the semantic content of the speech; *Self-adaptor,* in which the actor produced self-grooming hand movements that were not related to the story; *No-hand-movement,* in which the actor kept her hands in her lap; and *Non-visual* in which the story was presented as an audio stream only. Using the cortical activation data from each of these conditions, Skipper et al. were able to develop structural equation models to assess the impact and interconnectedness of each of the regions of interest on processing of speech and gestural information. Interestingly, Skipper et al. found that Broca's area was not as highly connected with other brain areas when processing co-speech gestures as would be expected based on the current understanding of mirror neuron theory. These unexpected results may have occurred due to the intention of the participants in this study, which was to closely listen to the speech produced by the actor. In the three conditions with visual components, participants were provided with visual information which was congruent with the speech stream. In the Gesture condition, participants viewed gestures that supported the speech, and in the Self-adaptor and No-hand-movement conditions participants were provided with visual information in the form of lip movements and facial expressions which also supported disambiguation of the speech stream.

Skipper et al. (2007) indicate that this may be reflective of the *dynamic* nature of the mirror neuron system, and actually demonstrates that activation of mirror neuron networks may be dependent on the intention of the observer/listener.

With the supporting visual information occurring in all three of the video conditions, ease of comprehension was comparable across conditions and the role of the Broca's area in action understanding (as part of the mirror neuron system) was reduced. The notion of a dynamically activated mirror neuron system is further supported by the finding that there are different patterns of activation when speech-associated gestures are present (in the Gesture condition) compared to when they are not (Self-adaptor, No-hand-movement). In the Gesture condition Skipper et al. report strong connections in activity observed across dorsal and ventral sections of pre- and primary motor cortices, the supramarginal gyrus and the anterior section of the superior temporal cortex, which is interpreted as a reflection of the mirror neuron system activating areas of the brain responsible for the execution of hand movements. In the two conditions when only facial actions were related to speech (Self-adaptor, and No-hand-movement) Skipper et al. report strong interactions in the pars opercularis, as well as ventral pre- and primary motor cortices, and anterior and posterior areas of the superior temporal cortex. Skipper et al. indicate that the role of the pars opercularis is in the matching acoustic and visual information about mouth movements with the motor plans responsible for producing those movements. They therefore maintain that this observation and comprehension of action (in this case mouth and tongue movements that produce speech) could also be reflective of mirror neuron activity, and similar to the observation of co-speech gesture, the observation of speech-related facial movements assists in understanding the speech stream. Furthermore, regarding the dynamism of the mirror neuron system, Skipper et al. posit that the different areas of the mirror neuron system of the observer are activated depending on the relevance of the observed action to the believed goal of the individual performing the action. In this case the goal of the performer was

communication of information (primarily via speech), and the regions of the observer's mirror neuron system were dynamically activated depending the relevance of the action (hand action or facial movement) to that goal.

Both the Willems et al. (2007) and the Skipper et al. (2007) studies reviewed above used stimuli based on the presentation of iconic co-speech gestures, which unlike emblematic gestures, have highly ambiguous, if any, meaning in the absence of speech. Understanding the neural outcomes of processing emblematic gestures is therefore also important. Gestural "emblems" are hand movements that can generally be understood in the absence of co-occurring speech (such as pushing the hand forward from the body with open flat palm facing toward the listener to indicate '*stop*'). Using observations from fMRI data, Lindenberg, Uhlig, Scherfeld, Schlaug, and Seitz (2012) investigated the brain regions activated during an emblem observation task. Participants watched a video portraying unimanual right-handed emblems and were asked to take the perspective of the gesture performer (the 'expression' condition) or take the perspective of the observer/receiver of the gesture (the 'reception' condition). Lindenberg et al. found that regardless of perspective condition, observing emblems led to increased activity in the left inferior frontal gyrus including the pars opercularis, pars triangularis, dorsal and ventral premotor cortex areas, as well as regions of the occipital lobe. When comparing perspective, they report that in the receptive condition, reduced activity was evident in premotor areas, and increased activation in the left pre-supplementary motor area and the anterior medial prefrontal cortex. Lindenberg et al. interpret much of their data in relation to the imagery that participants need to evoke to change 'perspective' in this task. However they do link their findings to mirror neuron theory and suggest that

this may provide further evidence of an action observation system that includes cortical regions homologous to the monkey F5 cortical area (Arbib, 2002).

When comparing emblem comprehension with the processing of other gestures, Villarreal et al. (2008) cite fMRI evidence that indicates that emblems evoke activity in distinct cortical areas. In that study, participants viewed a recording of an actor producing what they term 'transitive gestures' (gestures that mime an action such as using a screwdriver), 'intransitive gestures' (emblems like a salute), and meaningless hand movements. The authors found that observation of all types of gestures led to increased activity in right pre-supplementary motor area and bilaterally in the posterior superior temporal cortex, posterior parietal regions and occipital regions. Interestingly, they also found greater activation in the left inferior frontal gyrus, specifically the pars opercularis during the observation of intransitive/emblem gestures. Similar to Lindenberg et al. (2012), the authors cite activity in this area as being reflective of the mirror neuron system activation. When considering the neural correlates of emblems versus other gesture types, it is important to note that although intransitive gestures used in the Villarreal et al. study are clearly emblems, the transitive gestures (mimes) do not easily equate to iconic co-speech gestures (particularly since participants were not presented with a simultaneous speech stream) and therefore these findings should not be interpreted as comparing iconic gestures with emblems. Despite this, it does provide further insight into the cortical areas involved in processing emblems, and does provide further support for the existence of a mirror neuron system and its engagement in the processing of linguistically meaningful hand actions

While the above fMRI studies addresses the neural correlates of emblems, other researchers have sought to investigate the functional importance of emblems in

communication. Bernardis and Gentilucci (2006) examined variations in speech and hand movement in participants who were required to perform an emblem that described a word, speak that word, or both. Executing gesture and speech simultaneously led to changes in the acoustic properties of the speech that weren't present when speech was produced in isolation, or when it was accompanied by a meaningless hand movement. Furthermore, Bernadis and Gentilucci found that the duration of the hand-movement decreased when the speech and the gesture conveyed the same meaning, compared to gesturing alone, or simultaneously producing a meaningless hand movement with a meaningless word. Due to the focus on behavioural outcomes of emblematic gesture production, the results from this study are difficult to integrate or compare with the fMRI from the previously reviewed studies. Despite this, these results provide some indication that like iconic co-speech gestures, emblem production can influence 'regular' (or in this case, emblem-free) speech behaviour and vice versa.

To expand on this first study Gentilucci, Bernardis, Crisi, and Dalla Volta (2006) sought to use rTMS to explore the neural regions that might play a role in the processing of emblematic gesture. As mentioned earlier in this thesis, rTMS can be used to create temporary changes in cortical excitability. Many of these studies have focussed on the impacts of rTMS on motor cortex excitability due to the relative ease of which excitability in the motor cortex can be measured via EMG data collected from electrode sites at the target muscle groups. Fitzgerald, Fountain, and Daskalakis (2006) authored a comprehensive review on the inhibitory and excitatory effects of rTMS on the motor cortex. Broadly, the application of rTMS can be categorised by pulse frequency and can either be considered low frequency (<1 Hz) which can potentially induce inhibitory effects on cortical networks, or high frequency (> 1

Hz), which can potentially induce facilitatory effects on cortical networks. A variation of rTMS called theta burst TMS can be used to produce differential and long-lasting effects on cortical excitability following a brief period of application (Huang et al., 2005). Using rTMS, Gentilucci et al. sought to depress cortical activity in Broca's area in participants prior to a gesture and speech recognition task. In that study, six native Italian-speaking participants were required to identify three 'communication' words presented on screen in one of three modalities. Each word (*ciao*, *stop*, and *no*) was presented as text onscreen, or as a short video clip display of the face and torso of an actor who presented the word using speech or gesture. As a control, Gentilucci et al. also presented three 'colour' words which used similar initial vowel elements to the 'communication' words (*giallo* - yellow, *rosso* - red, and *rosa* - rose). Again these words were presented in text and speech and a coloured spot was digitally imposed over the gesturer's hand for the colour equivalent of the gesture modality. In all of these conditions, participants were required identify the word presented and speak their response. To best target Broca's area with rTMS, the authors sought to induce speech arrest using rTMS. The researchers identified a specific left hemisphere motor cortex location (using typical hotspot determination methodology) and then progressively moved the coil laterally and anteriorly applying short trains of rTMS at different locations. They reported that ideal intensity and location were determined when participant speech started to deteriorate during a concurrent counting task. Gentilucci et al. were cautious to point out that speech arrest in this case was non-motor related (i.e., not caused by interference with the motor cortical areas responsible for controlling speech musculature such as the muscles in the tongue and lips). Gentilucci et al. then applied rTMS at the intensity that elicited speech arrest for 300 pulses at a rate of 1Hz. Participants completed the

task on three occasions: after rTMS was administered over the target cortical area (Broca's area), after rTMS was administered over the homologous right hemisphere cortical area, and after sham rTMS was administered.

Due to the researchers' interest in language production, Gentilucci et al. (2006) reported the impact of rTMS on a range of speech quality components (such as vowel formation, and vocal pitch) as well as reaction time (RT). The authors found that during sham rTMS, RT to gesture and speech were both slower than RT to text, but did not significantly differ from one another. They also report that rTMS over Broca's area enhanced the delay for RT to speech stimuli, so that it was significantly slower than both text and gesture. As they report that rTMS had an impact on the spoken response to gesture stimuli (as measure by variations in lip movements during response), but no impact on RT, Gentilucci et al. suggest that there may be some interaction in motor networks that is causing the disruption to word formation that is not detectable in RT data. Specifically they hypothesise that seeing the gesture stimuli may trigger the execution of 'covert' rehearsal of the motor program for gesture in the observer while they are simultaneously trying to formulate/execute the motor program that allows their lips/tongue/larynx etc. to produce the response word (Gentilucci et al., 2006). While Gentilucci et al. don't specifically link this result to the activity of the mirror neuron system, this hypothesis would be well supported within the framework of mirror neuron theory.

As demonstrated by Gentilucci et al. (2006), the use of rTMS to temporarily impede function in Broca's area delays an individual's ability to process speech information but not gestural information, and the impact that rTMS application to other cortical areas has on gestures is unknown. Previous researchers have indicated that areas associated with the cortical motor system (Lindenberg et al. 2012; Villarreal et al.

2008) and specifically the motor cortex (Skipper et al., 2007) show distinct activation patterns during the processing of gestural information, perhaps a reflection of mirror neuron system activity. Similar to the rTMS 'virtual lesion' created in Broca's area in the Gentilucci et al. study, theta-burst TMS, specifically continuous theta burst stimulation (cTBS), can be used to create virtual lesions or reduce excitability in other cortical areas such as the motor cortex (e.g., Huang et al., 2005).

Aims and Hypotheses

The aim of the present study is to investigate potential effects of reduced motor cortical activity on gestural processing by comparing RT to stimulus words presented in four modalities: text, speech, gesture and combined speech and gesture. As the motor cortex is thought to be recruited during gestural processing (Skipper et al., 2007), it is anticipated that when theta-burst TMS is applied to the hand area of the motor cortex, gestural processing will be more difficult for the participant and RT will be delayed. Accordingly, it would also be expected that theta-burst TMS would only impact the processing of gesture-based stimulus, and would not affect the identification of text or speech stimuli. It is unclear exactly what impact cTBS over the motor cortex would have on stimuli that are presented simultaneously as speech and gesture, but it would be expected that the speech stream would provide enough information to sufficiently overcome any delays in processing gestural information. . It is therefore hypothesised that there would be no significant difference in RT to text, speech, or combined speech and gesture stimuli between cTBS and control (sham) conditions, and that delayed RT would be only be seen in response to gestural stimuli when cTBS is administered.

Method

Participants

Twenty four individuals volunteered to participate in this research. Following a screening session (detailed below) only ten participants were selected to continue. Participants were aged between 18 and 20 years (M = 19.1, SD = 0.57 years) and 5 participants were female. All participants were first year university students who were recruited through the School of Psychology at the University of Tasmania and were awarded four hours course credit for their participation. This research was granted ethics approval by the *Human Research Ethics Committee (Tasmania) Network (no. H0009812).*

Participants self-reported that they were right-handed and had no language or visual deficits. Participants completed a consent form and medical questionnaire at the beginning of the first session (Appendices B and D) and no participants reported contraindications to TMS (including; concussion, migraines, metal implants, pacemaker, or familial history of fits or seizures). All participants spoke English as their first language.

Apparatus

Stimulus

The stimuli words used in this experiment were *no*, *hello*, *stop*, *listen*, and *quiet*. These five words each have an associated emblematic gesture, that is to say a gesture that is used to assist in communicating the speech content, and has the potential to effectively communicate in the absence of speech (Rossini, 2012). The words and gestures chosen were based on those used by Gentilucci et al. (2006) (*no,*

hello, stop), with two additional words (*listen*, and *quiet*). All participants endorsed the gestures as being familiar and recognisably associated with the word. It was noted however that the some of the gestures could also be representative of other words (e.g., for *hello* a waving hand could mean '*goodbye*' or could be used to call an observer's attention, and similarly for *listen* the open palm forward facing fingertips behind the ear could mean '*speak up*'). To overcome this, before beginning, participants were familiarised with which of the associated words-gesture pairings were considered correct for this experiment. Participants viewed a video stimulus that presented each word in four different modalities: gesture with speech; gesture only; speech only; and text. In the text condition, words were presented one at a time in size 40 Arial font in white text on a black background (see Figure7.1a below). In all remaining conditions the monitor displayed an actor sitting at a table. To provide the most naturalistic gesture presentation, the video was framed to ensure that the actor's face and upper torso were visible. Each video clip was 3 seconds long and began with the actor's hands resting flat on the table. In the speech only condition, the actor spoke target words without moving hands from the table. Audio was recorded independently of video and identical audio streams for each word were dubbed in to the speech only and gesture with speech conditions to eliminate variance in speech patterns and volume. Due to the naturalistic nature of speech and gesture, it was determined that it would it would be unrealistic, and unreasonable, to attempt to ensure that latencies of gesture and speech were identical to the millisecond across presentation modalities. Speech onset, and stroke onset (when hand movements started to form an identifiable gesture) for each stimulus word are outlined below in Table 7.1. These times represent the onset target stimulus within

each video clip. In the combined speech and gesture condition, stimulus onset is taken from the emergence of the stroke phase of the gesture.

Table 7.1. *Onset of Stimulus for Words in Each Modality Following the Fixation Cross.*

	Text	Speech	Gesture	Speech and Gesture
Hello	0 ms	1000 ms	1100 ms	1100 ms
No	0 ms	1130 ms	1168 ms	968 ms
Stop	0 ms	1000 ms	1035 ms	1100 ms
Quiet	0 ms	1100 ms	900 ms	835 ms
Listen	0 ms	1000 ms	800 ms	800 ms

Note: Combined Speech and Gesture stimuli onset is calculated as the start of the stroke phase of the gesture.

Stimulus Presentation

In all conditions the stimuli were preceded by a white fixation cross on a black background that was presented for 300 ms. At 200 ms the participant also perceived a beep tone. The fixation cross was followed by the presentation of the stimulus which was either a printed word, an actor speaking or gesturing, or speaking and gesturing. Modality of word presentation was the same within each block of trials, and therefore each block only contained one presentation modality (e.g., gesture) and not a combination of modalities. The presentation of each word was followed by a variable ISI between 3 and 5 s (see Figure 7.1a and b). Within a block, each of the five words was presented ten times in a randomised order, equating to total of 50 trials per block. Participants wore a headset equipped with binaural headphones and a microphone. The associated audio stream was delivered via the headphones and the microphone was used as a response device.

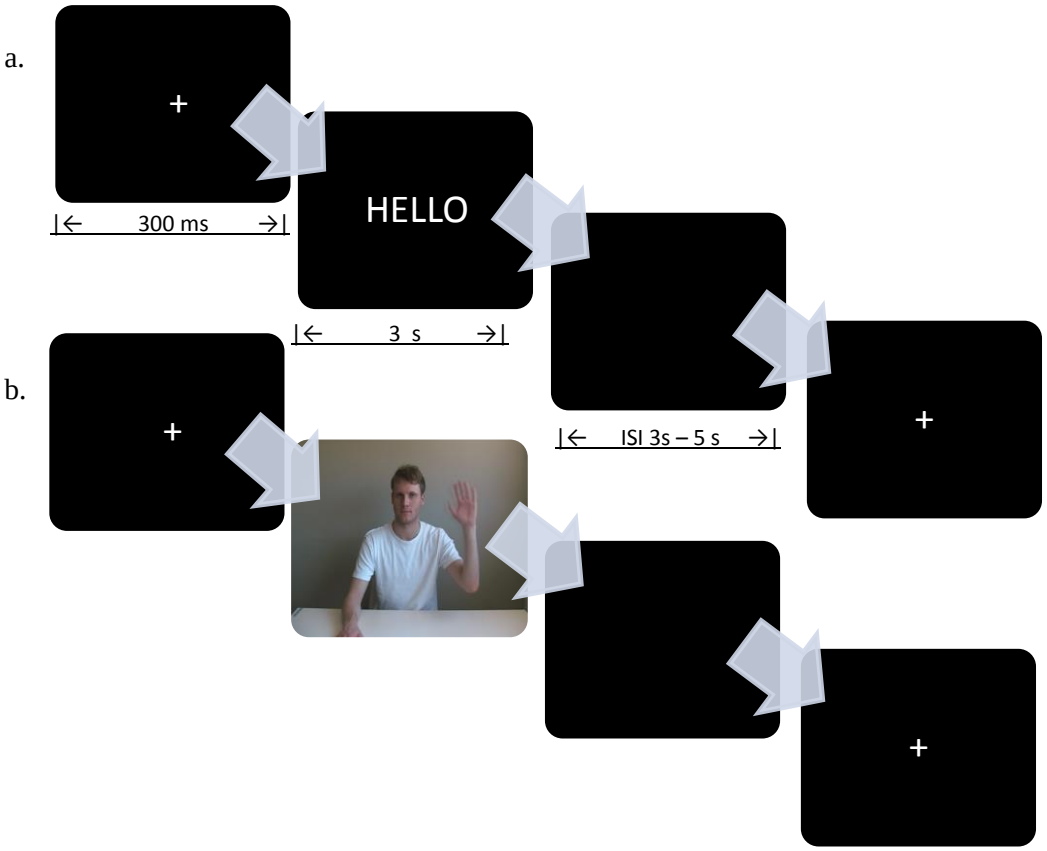

Figure 7.1a and 7.1b. Sample of timing of stimulus presentation for a) text stimuli and b) gesture stimuli.

TMS and EMG Recording Apparatus

Single-pulse TMS was applied using a Magstim 200 (Magstim Co., Whitland UK) stimulator through a figure-of-eight coil (70 mm diameter of each wing). cTBS was conducted using Magstim SuperRapid. EMG data were recorded from the first dorsal interosseous (FDI) muscle of the right hand. Ag/AgCl electrodes were attached at three sites –over the belly of the right hand FDI muscle, on the second metacarpophalangeal joint of the right index finger, and a reference electrode on the styloid process of the right radius. EMG activity was band-pass filtered (20 to 1000 Hz), amplified (X1000) using CED1902 amplifiers (Cambridge Electronics Design, Cambridge, UK) and then sampled using a CED Power1401 data acquisition system. Sweeps were collected 100 ms before to 3300 ms after the presentation of the

fixation cross using Signal 3.08 software (Cambridge Electronics Design, Cambridge, UK).

The microphone was attached to voice-activated relay to generate a TTL pulse that was used to record response onset.

Procedure

Participants attended three sessions (a screening session and two test sessions) within a laboratory at the University of Tasmania's Psychology Research Centre. The purpose of the screening session was to determine the applicability of the cTBS protocol for each participant. As this study is primarily focussed on investigating gestural processing when motor cortex excitability is depressed, it was important that participants who showed no reduction in motor cortex excitability following cTBS were excluded from continuing in the study. A more detailed explanation of the screening protocol and the excitability measures of the 'responder' participants is outlined below in the procedure section.

At the beginning of the screening session, participants were given a consent form, information sheet and a medical/history questionnaire to ensure their suitability for the study. The researcher gave participants an opportunity to ask questions at the beginning of and throughout the sessions. Participants were requested to wash their right hand and wrist with soapy water in order to remove excess dirt from the skin. An abrasive gel was then used to abrade the area of skin over the electrode sites. Alcohol swabs were then used to wipe away dead skin cells from these areas. Electrodes for EMG recordings were then attached.

Participants were seated in a comfortable adjustable chair approximately 60cm from the computer monitor. The electrodes were connected to the amplifier

and recording computer, and their outputs were assessed to ensure they were accurately recording muscle activity. Electrodes were adjusted if necessary to ensure conductivity, and participants were instructed to relax their muscles if involuntary motor activity was visible in the recordings.

TMS Procedure

The ideal location for application of TMS (or the "hotspot") was determined by holding the TMS coil over the motor cortex of the left hemisphere (approximately 5cm lateral and 1cm anterior of the midpoint of the head). The coil was repeatedly discharged as coil position and orientation were adjusted to find the scalp location that yielded a maximal MEP amplitude in the right FDI. Once the hotspot had been determined, an ink marker was used on the scalp of the participant to mark the position of the coil to ensure consistent coil placement across trials. Maintaining the coil over the hotspot, the researcher then determined resting motor threshold (RMT, defined as the minimum charge intensity needed to evoke at least 3 MEPs $\geq 50\mu$V on 5 consecutive trials). To determine baseline motor cortex excitability (prior to cTBS administration), recruitment curves were obtained using intensities of 110%, 120%, 130%, 140% and 150% of RMT. Ten MEPs were elicited at each intensity. Recruitment curves were also obtained immediately following cTBS (0 minutes) and again at 5 minutes, 10 minutes, 15 minutes, 20 minutes, and 25 minutes post cTBS. The mean MEP amplitude change (as a percentage of baseline) across selected participants ('responders') was calculated and collapsed across stimulation intensities is shown in Figure 7.2.

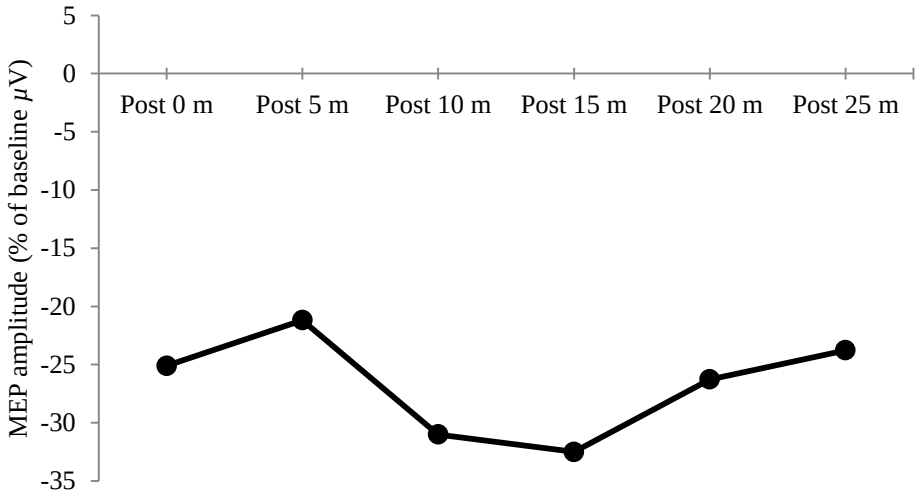

Figure 7.2. Percentage reduction in excitability for cTBS responders at all post-cTBS time points in the screening session. All responders showed reduction in MEP amplitude post-cTBS.

The cTBS protocol employed in this study , previously reported by Huang et al.(2005), consists of bursts of three pulses (at a rate of 50 Hz) delivered at 200 ms intervals at 80% of active motor threshold (AMT) over a period of 20 seconds (total of 300 pulses). This cTBS protocol has been shown to create a reliable depression in motor cortex excitability for approximately 20 minutes (Huang et al., 2005). Threshold for cTBS administration was determined independently from single pulse threshold due to the TMS pulses being delivered by a different TMS machine, Magstim SuperRapid, and the necessity for cTBS to be delivered at 80% of AMT rather than RMT. To determine AMT, participants were requested to perform a mild voluntary contraction of FDI by pinching their thumb and index finger together to elicit EMG activity approximately 50 μV peak-to-peak. Participants were provided with onscreen visual feedback to assist them in maintaining this level of motor activation. Single pulse TMS was then applied over the previously identified hotspot, and AMT was identified as the intensity at which TMS could evoke an MEP

amplitude of approximately 100 μV on 3 out of 5 consecutive trials during the voluntary contraction. Following determination of AMT, cTBS was then administered as per the above cTBS protocol at 80% of AMT over the left motor cortex. In the testing sessions (sessions two and three) participants underwent a similar cTBS setup procedure including the determination of hotspot, RMT and AMT. Unlike the screening session, the testing session setup contained no baseline/ongoing checks of motor cortex excitability. Due to the time-limited excitability effects of cTBS, it would be impractical to attempt to check excitability throughout experimental data collection in testing sessions.

Testing Session Procedure

Participants who showed a reduction in MEP amplitude following cTBS were invited to participate in two testing sessions. In each session, the experimenter administered an authentic cTBS or sham protocol, and the order of sessions was counterbalanced across participants. Using identical set-up protocol to the screening session, the experimenter determined hotspot, RMT, and AMT in both sessions two and three. In the session, cTBS was administered over the hotspot at 80% of AMT as indicated above. In the sham session, the TMS coil was angled perpendicular to the scalp effectively ensuring magnetic pulses were discharged away from the brain. As observed by Huang et al. (2005), reduction in excitability is observable after approximately 5 minutes post rTMS, and in this sample, reduction in excitability was maximal at post 10-15 minutes in the screening session (as shown in Figure 7.2). Prior to beginning the task, participants were informed of the five gesture word combinations they would view. The experimenter demonstrated each of the gestures and their accompanying words, and then demonstrated the gestures only and asked

the participant to identify each of them. The headset was adjusted to fit the participant's head and ears and the microphone was adjusted to ensure that it was accurately detecting voice responses.

In each block, participants viewed the 50 items, and were instructed speak the words as soon as they could identify them. Participants were encouraged to respond as quickly and as accurately as possible. The experimenter recorded the content of participant responses which were then later matched for accuracy, and voice RT was recorded via the Signal program. Participants completed four blocks (one for each modality). Each block ran for approximately 5.5 to 6.5 minutes. The order of blocks was counterbalanced across participants and across sessions to control for the dissipation of cTBS effects on excitability. Participants were contacted to arrange a third session at the closest convenience within 6 - 14 days after their second session (M = 8.4, SD = 3.03 days).

Design

This research employed a single independent variable experimental design with TMS (cTBS, Control) as the factor. This was repeated for four conditions of Word Modality (Text, Speech, Gesture, Speech and Gesture). The dependent variable was RT for correct responses.

Results

RT was measured as the time of onset of correct verbal response. Incorrect responses were omitted from the final data analysis. Additionally, as each word within each presentation modality had non-identical onset latency, RT was adjusted and was determined to be the time taken to respond after the onset of the stimulus. In the case

of the combined gesture and speech condition, RT is adjusted according to the onset of the stroke phase of the gesture. These variable stimulus onsets are outlined previously in Table 7.1. Table 7.2 below displays the means and standard deviations for each modality in both cTBS and Control (sham) conditions. The data in Table 7.2 show that RT is minimally slower when cTBS is present in both the Text and Speech conditions, whereas RT is minimally faster when cTBS is present for Gesture and the combined Speech and Gesture conditions. Considering the high standard deviations, the variance in the data combined with the minimal difference in means indicates that there is unlikely to be a significant difference between cTBS and sham conditions and potentially a small effect size.

Table 7.2.

Means and Standard Deviations (In Parentheses) for RT (in ms) in Each Presentation Modality.

Presentation condition	cTBS	Control
Text	736.14 (92.30)	724.14 (90.36)
Speech	741.02 (190.47)	737.70 (168.09)
Gesture	858.03 (115.76)	860.28 (100.55)
Speech and Gesture	743.78 (157.41)	767.18 (166.60)

Separate one way analysis of variance (ANOVA) tests were conducted to examine potential differences in RT for each presentation condition. It was found that there was no main effect of TMS in any condition as the differences in RT did not reach significance in the Text condition, F (1,9) = 0.98, p =.35, η^2_p = .099, g = 0.13, Speech condition, F (1,9) = 0.02, p = .89, g = 0.02, Gesture condition, F (1,9) = .004, p = .95, g = 0.02, or Speech and Gesture condition, F (1,9) = 0.90, p = .37, g = 0.14.

Discussion

The aim of the study was to determine the impact that depressing motor cortex excitability would have on the speed at which participants process gestural information. It was expected that reduced motor cortex excitability would increase the amount of time required to process gestural information, whereas modalities that did not rely on gestural information for comprehension would not be impacted by cTBS. It was therefore hypothesised that when motor cortex excitability was depressed, RT would be significantly slower for Gesture stimuli in cTBS condition compared to control, and there would be no difference in RT between cTBS and Control (sham) for the other three modalities. As expected cTBS had no effect on the processing Text, Speech, or combined Speech and Gesture stimuli, however contrary to expectations cTBS also had no significant effect on RT to Gestures.

Interpretation of these results gives rise to several plausible explanations. Firstly, they could be interpreted as evidence against the idea that the motor cortex is involved in gestural processing, or perhaps more specifically they could be evidence that the motor cortex is not involved in the processing of gestural *emblems*. A recent article by Andric et al. (2013) published after the conclusion of data collection for the current study provides additional insight into cortical activity during the processing of emblems. Andric et al. used fMRI data to identify brain areas activated during emblem observation and compared this to listening to speech or observing a grasping movement. The authors theorised that whilst grasping observation (a motor movement) and speech (a medium containing symbolic meaning) would activate distinct cortical regions, the observation of an emblem (a motor movement with symbolic meaning) would activate areas common to the processing of both speech and movement observation. They found that many cortical areas are activated by

both speech and observation of emblems, more specifically the right posterior mediotemporal gyrus and anterior superior temporal gyrus, and the left pars opercularis and pars triangularis. Andric et al. report that these areas are activated during the processing of meaning regardless of whether it is presented in the form of speech or an emblem. They also observed an increase in neural activity in the bilateral dorsal and ventral premotor areas (as well as intraparietal sulcus and superior parietal cortices) for both observation of emblems and grasping compared to speech, indicating that these areas are activated by movement observation, but not necessarily involved in the processing of linguistic meaning, at least not in the same way as the cortical activation that overlaps speech and emblem processing. Relating back to the current study, it is therefore possible that while motor cortex activity was depressed, this may have only impacted the processing of the movement observation, as areas responsible for processing the linguistic meaning of emblems (such as the pars opercularis and pars triangularis) were unaffected by the application of cTBS.

An alternative explanation is that depressing the motor cortex using a cTBS protocol does not sufficiently reduce motor cortex activity to the point where it impacts gestural processing. Previous authors have already noted that while the application of cTBS may create changes in motor cortex excitability (usually measured via changes in MEPs), these changes in excitability do not necessarily translate to behavioural changes. For example, Rossi et al. (2000) found that while there were reductions in cortical excitability following 15 mins of low frequency (1Hz) rTMS, there was no impact on participant performance in a finger tapping task. Ridding and Rothwell (2007) discuss the potential for homeostatic-like after effects of rTMS. That is, when a cortical area is temporarily depressed by rTMS, the brain is able to 'compensate' and rapidly engage alternative cortical networks to

perform the task. Lee et al. (2003) administered 30 minutes of low frequency (1Hz) rTMS over the primary motor cortex. PET data were collected before and after rTMS during rest and during a finger tapping and free movement tasks. It was found that despite the fact that there was no impact of rTMS on any of the behavioural outcomes of the hand movement or finger tapping tasks, there were widespread changes in task-related cortical activity following rTMS. Lee et al. report that while the primary motor cortex became less responsive to input from premotor and medial motor areas, there was increased dual activation of the inferior medial section of the left motor cortex and anterior motor areas. Lee et al. refer to the ability of the brain to 'remap' cortical networks activated to complete a task following a virtual lesion as 'acute compensatory plasticity'. Ridding and Rothwell claim that this compensatory response may explain why changes in cortical excitability often do not create change in behavioural motor tasks (as demonstrated by Rossi et al., 2000). While it must be acknowledged that the homeostatic effect outlined above is related to a motor task, it is possible that in the current study, the lack of change to RT does not accurately measure change in the motor cortex's role in gestural processing, and that the lack of significant behavioural effects may be due to the ability for the brain to utilise alternative processing pathways to rapidly compensate for the rTMS induced virtual lesion. Unfortunately without access to corroborating brain imaging data, it is not possible to confirm this hypothesis.

There are some methodological limitations that need to be considered when interpreting these results. First is the reliability of cTBS creating similar impacts on motor cortex function excitability over multiple administrations. In this study, although participants were initially screened so only cTBS responders (those showing depressed motor cortex excitability) were recruited. Due to the short-lasting

effects of the cTBS protocol used in this study and the resulting necessity to quickly proceed with the experimental data collection, excitability was not re-checked in subsequent testing sessions. As covered in the TMS chapter (Chapter 5) there is only one very recent study that has examined the variability within individuals over multiple sessions. Vernet et al. (in press) reported high levels of intra-individual variability across two sessions of cTBS testing. Therefore in the current study, it is possible that the reduced excitability seen in the initial screening session may not have occurred in the cTBS testing session, or may have occurred to a lesser degree.

In addition to lack of certainty about whether or not cTBS operated as expected on motor cortex excitability in testing sessions, there are a number of other limitations. The size of the participant sample who completed this study was quite small (n = 10), which is largely due to the limited number of recruited participants who were identified as responders to the cTBS procedure. Despite the fact that the experiment that the current methodology was based on only had six participants (experiment two from Gentilucci et al., 2006), a larger sample may have improved the robustness of these results. However, based on how similar the RTs were between modalities, and the small effect sizes it would be unlikely that a larger sample group would lead to significant RT differences between stimulus types. Another difference between this study and that of Gentilucci et al. is the number of times each stimulus was shown. In the Gentilucci et al. experiment, participants only viewed each stimulus word five times in each modality. It is possible that in the current study, displaying each word ten times may have led to participants becoming too familiar with the stimuli and being able to pre-empt the upcoming stimulus word based on extraneous visual information contained in the clip, such as small changes to facial expression, lip configuration, pre-stroke phase hand movements. It could

also be argued that the increased number of stimulus presentations used in the current study creates more robust measure of RT, and should not be considered a methodological flaw. That said, familiarity with the stimuli may have allowed participants to predict the upcoming word, meaning that RT may be a reflection of reaction to non-linguistic visual information (as above) rather than linguistic information contained in the actual speech stream or gesture. This obviously limits the interpretability of any effects (of lack thereof) and their connection with the application of cTBS over the motor cortex. To remedy this and maintain the high number of repetitions in future research, it would be possible to redesign the stimuli so that the face is occluded. This would limit extraneous information and speech could be dubbed in as necessary.

In summary, through the findings of this study, it has been demonstrated that a cTBS generated virtual lesion over the left hemisphere motor cortex produces no behaviourally identifiable impact on the processing of emblem gestures, either independently performed or co-occurring with speech. In turn, this study provides no evidence to support the notion that the motor cortex is involved in the processing of gestures (specifically emblems) however, as noted above, this does not necessarily mean that the motor cortex is not involved, but that the cTBS protocol used was ineffective at producing changes to reaction time, even if it reliably changes excitability. This highlights the need for further research involving the employment of methodologies that offer potential sources of convergent evidence (such as neuro-imaging).One source of converging physiological evidence that has been used in gesture studies is EEG (e.g., Kelly et al., 2010). Using EEG data to provide more insight into the role of the motor cortex in gestural processing is explored further in Study Three (Chapter 8).

Chapter 8: Study Three

Introduction

The N400 component of ERPs found in EEG data has been repeatedly cited in papers reporting on semantic integration (Bentin, McCarthy, & Wood, 1985; Kelly et al., 2010; Kutas & Hillyard, 1980, 1984, 1989; Kutas, Neville & Holcomb, 1987;). In a landmark study, Kutas and Hillyard (1980) investigated the ERP components that were altered by the processing of incongruent linguistic stimuli. Participants viewed a series of sentences that either contained target words that semantically mismatched the overall meaning of the sentence (semantically incongruent) or target words displayed in an unusually large font (visually incongruent). They reported an increase in amplitude of a late negative component (the N400) only when target stimuli were semantically incongruent. In a subsequent study, Kutas and Hillyard (1984) found that while unexpected sentence endings reliably led to a larger N400 amplitude, the component was smaller when the incongruent terminal word was related to the expected sentence ending (e.g., 'He liked lemon and sugar in his *coffee*' instead of '*tea*'). Furthermore, it was also reported that whole sentences are not necessary to semantically prime a participant in order to elicit an N400; results from follow-up studies indicated that N400 amplitudes could also be evoked by single word lists that were presented outside of the context of a sentence (e.g., presenting a priming word '*doctor*' followed by a semantically congruent word '*hospital*' or incongruent word '*parrot*') (Bentin, et al., 1985) and simultaneous presentation of congruent or incongruent word pairings (Kutas & Hillyard, 1989).

Kutas et al. (1987) report that congruency-linked N400 amplitudes can also be elicited by auditory and sign-language stimuli. Kutas et al. examined EEG data from three groups of fluent English or American Sign Language participants who were presented with either printed English sentences, auditory English sentences, or American Sign Language sentences. They found that when presented with incongruent stimuli, N400 amplitude was larger across all three groups. Kutas et al. interpret the persistence of N400 effects across the three groups as evidence that the N400 is not merely a reflection of translation of visual/auditory information into phonetic components, and that it is more likely due to attempts to integrate semantically challenging linguistic information presented in all three modalities. Ultimately, researchers such as Kutas and Hillyard (1984,1989) conclude that the N400 is reflective of the integration of semantic information, and the greater the semantic distance between the expected target and the actual target, the greater the negativity of the component. Hagoort, Baggio and Willems (2009) advance the notion of semantic integration, and they suggest the N400 reflects part of a process they call 'semantic unification'. They describe this as a continuing process in which "discourse models (are) built up incrementally (and) at any one stage may have to be revised when additional information becomes available" (p.6).

Debruille (2007) disagrees that the N400 is a marker of integration processes, and instead argues that the increased amplitude seen in semantic incongruency studies is actually a reflection of cortical inhibition processes. Debruille proposes that the presentation of priming stimuli creates spreading activation throughout association networks which then needs to be inhibited following the subsequent presentation of a non-compliant stimulus. Debruille cites evidence from observations that found N400 amplitude can also be modulated by non-semantic linguistic

sentence structure violations. DeLong, Urbach and Kutas (2005) manipulated the terminal word as well as the 'a/an' article within sentences such as 'The day was breezy so the boy went outside to fly ... a kite / an kite / a airplane / an airplane". DeLong et al. found greater N400 amplitude to both 'an' and 'airplane'. Drebruille argues that as the a/an article holds no semantic relationship to the expected ending to the sentence (in this case 'kite'), the modulation of the N400 amplitude in this example may be reflective of inhibiting activation rather than integrating semantic information.

In light of varied results from ERP research, despite many indicators that N400 is modulated in semantic integration paradigms, Kutas and Federmeier (2011) report that it is not entirely clear what actually drives the N400. In their review article, Kutas and Federmeier suggest that many previous interpretations of N400 effects are based on the assumption of a 'feedforward' comprehension processes. That is to say that words are first analysed as "perceptual objects and then as linguistic objects, culminating in a match between a phonological or orthographic input and a representation in the mental lexicon - i.e., word recognition" (p.638). Once recognised, accessing related semantic information is then prioritised, and the word can then be integrated into the broader mental understanding of its context (e.g., passage of text, or conversation). Kutas and Federmeier also provide evidence that the N400 may also be sensitive to 'pre-semantic' information. For example, Deacon, Dynowska, Ritter, and Grose-Fifer (2004) found distinct N400 effects when participants were exposed to pseudowords, which theoretically should not semantically activate linguistic networks like known words would. Kutas and Federmeier argue that in cases like this, low level word characteristics (such as orthography) are effectively pre-semantic in processing complexity and that the

N400 is being at least partially driven by some other function. The authors go on to propose that the N400 may actually be reflective of activity in a long-term memory system that is differentially activated by different stimuli. This theory somewhat accounts for the many variations in results as it allows for differences in external influences (e.g., stimuli context) as well as internal influences (e.g., participant's prior linguistic/semantic knowledge). Therefore, Kutas and Federmeier also state that the N400 can also "be modulated by factors that affect the input stream or the configuration of activity in semantic memory" (p.641), such as attentional processes (e.g., voluntarily attending to specific aspects of the stimuli).

As indicated above, N400 amplitude has been found to fluctuate based on experimental manipulations (such as stimuli congruency), as well as the modality of presentation of stimuli. Holcomb, Coffey, and Neville (1992), found that when sentences with congruent/incongruent terminal words were presented in both auditory (speech) and visual (text) modalities, the distribution of N400 amplitudes across the scalp differed between the two modalities. Holcomb et al. found that N400 amplitude was larger for visual stimuli compared to auditory stimuli over the parietal and temporal regions of the left hemisphere, whereas the stimuli from both modalities elicited similar (although larger) N400 amplitudes in the right hemisphere. Regarding scalp distribution, there is evidence to suggest that different stimulus types lead to different patterns of activation across the cortex. For example, visually presented word stimuli are more likely to elicit N400 peaks more medially and at centroparietal areas, and have been found to show a larger (more negative) amplitude in the right hemisphere compared to the left (Holcomb et al. 1992, Kutas & Hillyard, 1982). N400s to auditory word stimuli are more likely to evoke more central N400 peaks (Holcomb & Anderson, 1993), whereas N400 to co-speech

gestures have been observed maximally in central and parietal areas (Kelly et al., 2010). In a review of scalp distribution literature, Federmeier and Laszlo (2009) are careful to point out that the amount of information about the location of the underlying neural sources of the electrical activity must be interpreted cautiously, as source detection through ERP data is focally limited. Other researchers who have combined advanced ERP and fMRI data have been able to provide evidence linking ERP data with a specific site in a language processing study, and while these appear to be in regions not directly beneath the EEG recording site, they are proximal enough as to be found within the same hemisphere (Vitacco, Brandeis, Pascual-Marqui, & Martin, 2002).

Interestingly, despite many indications that the latency of the N400 is largely fixed, Federmeier and Laszlo (2009) report that there are very few known factors that influence the latency of the N400 component. Beyond neurological or psychiatric disorders such as schizophrenia or Alzheimer's disease (for a review see Federmeier and Laszlo, 2009), there is evidence that thus far only participant age and language dominance have been found to impact the timing of the N400 component. Regarding age, N400 latency has been found to increase at a rate of approximately 1.5 - 2.1 ms per year from 20 to 80 years of age (Kutas & Iragui, 1998). Regarding language dominance, Moreno and Kutas (2005) report that in a study comparing Spanish-English bilinguals, the N400 component had a delayed latency (approximately 27 ms) in response to non-dominant language stimuli compared to dominant language stimuli. Moreno and Kutas interpret this as evidence that it takes longer for participants to appreciate the semantic incongruency in a non-dominant language, leading to a delayed N400 component, a notion which they link with both vocabulary knowledge as well as age of second language acquisition.

As indicated above, some researchers have reported that this N400 effect extends to the integration of linguistic stimuli beyond written text. The N400 peak amplitude data has also been reported in studies where participants are required to simultaneously process gesture and spoken linguistic information (Cornejo et al. 2009; Kelly et al., 2004, Kelly et al., 2010; Özyürek, Willems, Kita, & Hagoort, 2007).

As reviewed earlier in this thesis, Kelly et al. (2004) examined ERP components that are augmented by the processing of semantically incongruent gestures. Participants viewed clips of an actor gesturing to physical feature of an object (e.g., gesturing to indicate the thinness of a glass) while speaking a descriptor word that was matching (e.g., "thin") or mismatching (e.g., "wide") to the gesture. Additional conditions in which the speech matched the object, but not the gesture (complementary) and a speech only (no gesture) condition were also included. Kelly et al. reported that incongruent (mismatching) speech-gesture pairings elicited larger N400 amplitudes than congruent (matching) pairs. The authors interpreted this as evidence that the integration of gesture and speech information occurs in the same late period (approximately 300-600 ms) as that of written semantic information integration (Kutas & Hillyard, 1980). A example of an ERP waveform that shows the N400 component from Kelly et al.'s paper is shown below in figure 8.1.

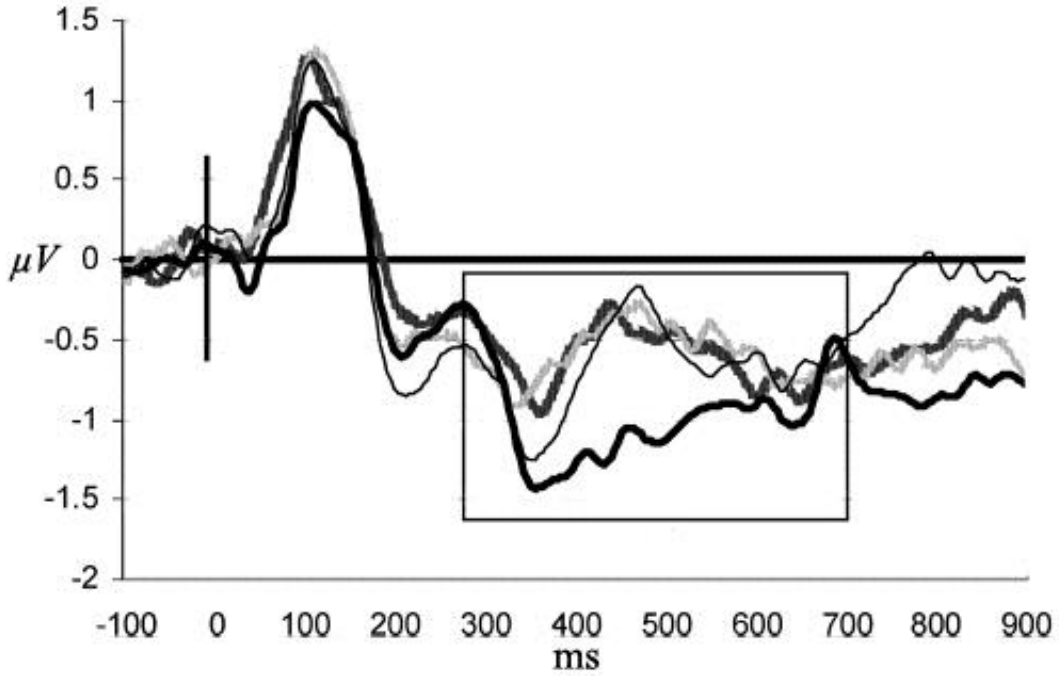

Figure 8.1. Adapted from Kelly et al. (2004, p.256) this figure shows a distinct negative component in the rectangular box. This component is most negative in the mismatching condition (the thick dark line)and occurs within the window expected for an N400 component.

Özyürek et al. (2007) broadened the naturalistic nature of the experimental stimuli by presenting participants with whole spoken sentences which included a critical verb that was presented with a gesture (e.g., "He slips on the roof and *rolls* down" while making a rolling gesture with the hands). The researchers manipulated the gesture and the target words so that the sentence either ended semantically congruently (as above) or incongruently (e.g., "...*walks* to the other side"). The researchers established four conditions: gesture either matches the speech and target words (as above); so that the gesture mismatches the target word and sentence (e.g., target words are "*rolls* down" while gesture indicates walking), so the gesture matches the sentence but mismatches the target word (e.g., target words are "*walks* to the other side" while gesture indicates rolling); or so that the gesture matches the target word but mismatches the start of the sentence (e.g., target words are "*walks* to

the other side" while gesture indicates walking). Özyürek et al. found greater negativity in the N400 component in all three of the mismatching conditions compared to the matching condition, but no significant difference in amplitude between the mismatching conditions. The authors conclude that this is evidence that regardless of the type of mismatch, the augmentation of the N400 component reflects the integration of language happens at the late stage and is evident for gestures even when presented in the context of a sentence.

In line with the notion that co-speech gestures are produced in order to assist the communication of the broader meaning of a message (McNeill, 2007; Wu & Coulson, 2005), Cornejo et al. (2009) conducted an ERP study examining the role of gestures in the understanding of metaphor. Specifically, the researchers were interested in checking whether N400 amplitude was augmented during the integration of mismatching gestures during a somewhat more naturalistic speech stream than the single-word/single-gesture pairings used in previous studies (e.g., Kelly et al., 2004), and during a the presentation of gestures that are more advanced than mere pantomime of an action described in a sentence (e.g., Özyürek et al., 2007). In the Cornejo et al. study, native Spanish-speaking Chilean participants viewed recordings of an actor speaking a metaphorical phrase such as "*esos vendedores son loros*" ("those salesmen are parrots") and gesturing simultaneously (e.g., closing extended fingertips to the thumb repeatedly to indicate talking too much) on the final word of the sentence. Importantly the video presentation was manipulated so that the gesture presented with the final word was either congruent to the metaphor being presented as in the example above, or incongruent, such as pointing toward the ground to indicate the present time while saying "*esos proyectos son historia*" ("those projects are history"). Cornejo et al. report that the N400

component displayed increased negativity in the incongruent conditions, demonstrating that gestural information is utilised when forming a metaphorical interpretation of speech stimuli. It could be said that this result was largely to be expected, as the mimed nature of many co-speech gestures are inherent metaphorical representations of the speech that accompanies them (Rossini, 2012).

More recently, Kelly et al. (2010) conducted a study examining the automaticity of the integration of speech and language information. Although that paper is reviewed more comprehensively elsewhere in this thesis, the methodology of the current study is based largely on that used in the Kelly et al. paper and therefore a brief review is warranted. Kelly et al. had participants watch a series of short video clips of an actor concurrently speaking single words and producing accompanying gestures. The video recording was framed around the actor's arms and torso. With the actor's face out-of-frame, participants could not glean any linguistic information from lip or tongue movements, and the researchers were able to manipulate the timing and the content of the speech stream without changing the visual stimuli. The occlusion of the presenter's face is important to note as in their previous study Kelly et al. (2004) had only demonstrated N400 gesture incongruency effects when participants were able to see the actor's face. In the more recent 2010 study, Kelly et al. manipulated not only the congruency of the speech stream that accompanied the gesture, but also the sex of the voice producing the words. In this way participants heard speech that either matched or mismatched the gesture, but also heard a voice that matched or mismatched the actor. Participants were simply asked to make a judgement of whether the sex of the actor matched that of the voice they heard, and were not required to make any active effort to semantically match gesture and speech. Kelly et al. (2010) report larger N400 amplitudes and slower

voice identification when the gesture and speech were incongruent. The authors concluded as the processing of incongruent speech-gesture information caused a response delay in the unrelated voice identification task, that the speech and gesture must be processed automatically.

Other researchers have examined other potential non-ERP neurophysiological changes that occur during linguistic processing. Seyal, Mull, Bhullar, Ahmad, and Gage (1999) hypothesised that corticospinal excitability may be modulated by the anticipation of performing a linguistic task. In that study, participants viewed a warning light, and then heard a go-signal tone before reading aloud or silently a word presented on screen. In other conditions participants were required to say the word 'cat', purse their lips silently, or not respond at all. Using single-pulse TMS over the hand area of the motor cortex, Seyal et al. measured changes in corticospinal activity at multiple latencies during the time period between the light and the tone, and in the period following the tone in each condition. The researchers observed that when the participant was anticipated reading aloud, larger MEP amplitudes where recorded during the task compared to rest. This effect was seen maximally in the left hemisphere but was also observed in the right. While it might be suggested that greater MEP amplitudes were due to activation of facial musculature in this condition, Seyal et al. report no hemisphere-specific MEP amplitude changes in the lip-pursing condition, indicating that the enhanced left hemisphere activation was unique to the condition in which participants anticipated reading aloud. Interestingly, the authors note a particularly "pronounced enhancement of TMS-induced amplitudes around 400 ms following the word stimulus" (p. 428). Although the suggestion that there is an underlying common neurophysiological mechanism at work in this case may seem too farfetched based

simply on the temporal overlap and linguistic nature of the task, Seyal et al. do go on to propose that like the N400 component, corticospinal excitability could potentially be sensitive to anticipation of performing a linguistic task.

Like Seyal et al., other researchers have speculated on the possible role of cortical motor systems in linguistic processes, particularly the comprehension of gesture. As covered in a Chapter Three, researchers such as Lindenberg et al. (2012), Skipper, et al. (2007), and Willems et al. (2006) have examined fMRI data while participants have performed gesture comprehension-related tasks. In all of these papers, the authors report activity in cortical regions that are responsible for motor control including the pre-motor and supplementary motor areas. Skipper et al. specifically found gestures that contained meaning related to a concurrent speech stream (iconic gestures) activated the primary motor cortex as well as surrounding motor cortical areas. Skipper et al. attribute the cortical motor system activity in this task to the operation of the mirror neuron system. Along with previous researchers who have hypothesised on the role of a mirror neuron system in the human brain (Rizzolatti & Craighero, 2004), Skipper et al. hypothesise that it is the meaningful nature of gestures that stimulates mirror neurons.

Even within the framework of mirror neuron theory, the role of the motor cortex in processing gestural information is still unclear. Both of the previous empirical studies conducted in this research paper relied largely on the impact of TMS on reaction time (RT) to investigate the links between language and the motor cortex, and thus far there has not been a comprehensive examination of other relevant neurophysiological evidence. Furthermore, while both studies required participants to make an assessment of the stimuli (i.e., real-word judgement and word-identification), neither of these studies required participants to make any

greater judgement on the semantic nature of the words or gestures presented. Additionally, the current study employs the use of iconic co-speech gestures, as contrasted with the emblematic gestures employed in the previous study. Iconic gestures were chosen for the current study due to their interpretation relying on a concurrent speech stream (Hadar & Pinchas-Zamir, 2004) and for their broad utilisation in the literature investigating N400 effects arising from speech-gesture integration (Cornejo et al. 2009; Kelly et al. 2010; Özyürek et al., 2007).

Aims and Hypotheses

The broad goal of this study is to further explore the role that the motor cortex may have in processing gestural information. Specifically, this study aims to investigate how the application of rTMS, specifically continuous theta-burst stimulation (cTBS), to reduce motor cortex excitability may yield effects that can be seen in recordings of brain activity during a gesture comprehension task. As indicated above, the amplitude of the N400 ERP component can potentially be augmented when the brain attempts to integrate semantically incongruent information (Kutas & Federmeier, 2011), an effect which also be been reported when integrating mismatching gesture and speech information (Kelly et al., 2004; Kelly et al., 2010). If the motor-cortex truly does have a role in the processing of gestural information, it is possible that when motor-cortex activity is suppressed (via an cTBS induced 'lesion'), the integration of gesture and speech information would be impacted. Therefore it also follows that the impact of reduced motor cortex activity on gestural processing may be reflected in neurophysiological measures, such as the N400.

It is difficult to precisely predict the impact that reduced motor cortex excitability would have on the N400 during a speech-gesture integration task. Regardless, it is anticipated that if the motor cortex is involved in gesture processing, the integration of gesture and speech would be impacted, and this would be reflected in changes in N400 amplitude. Despite an evolving understanding of the processes underpinning the N400 component (Kutas & Federmeier, 2011), it is generally accepted that the N400 component amplitude varies based on the degree of mismatch between the stimuli being processed. Integrating stimuli that are semantically distal are more likely to elicit greater N400 peaks (Kutas & Hillyard, 1984, 1989). Theoretically, it is not implausible that the integration of semantically distal stimuli could be viewed as a task which requires increased cognitive effort. If this 'effort hypothesis' is accurate, with reduced motor cortex activity the processing of gestural information may be more difficult, thereby increasing the demand on cortical resources needed to integrate the information resulting in increased N400 amplitude.

Alternatively, it is possible that a cTBS-induced temporary 'lesion' of the motor cortex could create a 'neglect' towards stimulus that it would usually process. Ellison, Schindler, Pattison and Milner (2004) have previously shown that rTMS of the right posterior parietal cortex can cause neglect-like symptoms (akin to that of a real lesion in the same area) in participants performing a visual search and visual perception task. While the application of this to cTBS of the motor cortex and potential impacts on the N400 amplitude may not be immediately apparent, it is possible that cTBS of the motor cortex may create a similar 'neglect' to gestural information. This could potentially lead to prioritising the speech information and reducing the importance of processing gestural information. If this "prioritisation hypothesis" is accurate, there would be reduced competition for integrating

mismatching stimuli, potentially reducing the amount incongruency attended to by the participant leading to a smaller N400 amplitude.

Regardless of the above, it is expected that semantically mismatching speech-gesture stimuli and semantically mismatching sentence stimuli (used as a control) will elicit larger N400 amplitudes than their semantically matching counterparts. Regarding the impact of cTBS on the N400, despite the tenuous nature of the "effort-hypothesis", if it is accurate, it is hypothesised that when cTBS has been administered over the hand-area of the motor cortex, the difference in N400 amplitude between congruent and incongruent speech-gesture stimuli is expected to be greater compared to when cTBS is not administered, with greater negativity observed in the incongruent condition. As suppressed motor-cortex activity should not impact processing of non-gestural printed sentence stimuli, it is also hypothesised that the difference in N400 amplitude for semantically incongruent sentence stimuli will be similar across cTBS and control (sham-cTBS) conditions.

Regarding latency of the N400 peak amplitude, it is largely unknown how latency might differ between the presentation of speech-gesture stimuli and sentence stimuli. As previous authors have reported that the latency of the N400 component is largely unaffected by task manipulations (see Federmeier & Laszlo, 2009, for a review), it is hypothesised that there will be no significant difference in N400 peak latency between gesture stimuli and sentence stimuli. As Moreno and Kutas (2005) report that a delay in recognition of incongruent stimuli is likely to lead to a delay in N400 latency, it is suspected that other factors which may delay this integration process may also result in a delay in latency. It is therefore expected that if cTBS reduces the effectiveness of the motor-cortex's ability to process gestural information, then N400 latency to gestural stimuli should be delayed when cTBS is

present. Accordingly, it is hypothesised that following application of cTBS, N400 latency will be delayed for gesture stimuli compared to control (sham-cTBS), and that this effect will only be observed in response to gesture stimuli as cTBS should not impact the processing of sentence stimuli.

Regarding the impact that cTBS of the motor cortex may have on reaction time (RT) in this task, while no previous literature provides a specific direction, other rTMS and language studies go some way to provide a plausible expectation. Oliveri, Romero, and Papagno (2004b) found that rTMS of the left temporal cortex led to delayed reaction time (RT) in a written idiom comprehension task, compared to rTMS of the right temporal cortex and no TMS. The authors concluded that the left temporal cortex is important in idiom comprehension based on the observation that an rTMS evoked virtual-lesion in that area of the brain delayed processing. Based on this, it is therefore predicted that in the current study, when motor-cortex excitability is reduced, integrating mismatching (incongruent) information will take longer than when the motor cortex is unaffected. Additionally, as identification of mismatching pairs is a more complex task, it is also anticipated that reaction times will be shorter when identifying congruent speech-gesture pairs than when identifying incongruent pairs.

Method

Participants

A total of 25 participants volunteered to take part in this research. This sample was distinct from that recruited for Study Two. Subsequent to a rigorous screening session (detailed below) only ten participants were selected to continue. Participants were aged between 19 and 34 years (M = 25.40, SD = 5.04) and six of

the participants were female. All participants were recruited through advertisement posters in the School of Psychology at the University of Tasmania. Participants were financially compensated for their time ($20AUD per session). This research was granted ethics approval by the *Human Research Ethics Committee (Tasmania) Network (no. H0009812)*.

Participants self-reported that they were right-handed and had no language or visual deficits. Participants completed a consent form and medical questionnaire at the beginning of the first session (Appendices C and D) and no participants reported contraindications to TMS (including; concussion, migraines, metal implants, pacemaker, or familial history of fits or seizures). All participants spoke English as their first language.

Apparatus

Gesture Task

In the Gesture Task, participants viewed 50 short video clips showing a presenter performing hand gestures of single words, supported by an audio track of the presenter speaking each word. In each clip the presenter is seated behind a white table in front of a blank mid-grey wall. The presenter is shown in the centre of the picture with full view of her upper torso, arms, and hands. The presenter's face cannot be seen as the picture is framed vertically from under the presenter’s chin to partway across the table surface, and framed horizontally approximately 40cm on either side of the presenter. See Figure 8.2 for sample video frames. Video clips were recorded using a tripod-mounted Sony Handicam and the speech stream was recorded using the Voice Memo application on an Apple iPhone 5. Voice and video

were subsequently edited on PC using the Sony Vegas Movie Studio suite of editing applications.

Figure 8.2. Sample frames from the beginning middle and end of 1 s clips for gestures '*close*' and '*saw*'.

A white fixation cross on a black background appeared in the centre of the screen for 100 ms before each clip. Each video clip played for 1000 ms, with the stroke portion of the gesture initiating at the start of the clip, and speech onset at 200 ms. Following the presentation of each clip a black screen was shown for variable ISI of between 1.5 s and 2 s. The list of words spoken and associated manual hand gestures used in this study are detailed in Table 8.1, and an outline of the presentation procedure is shown in Figure 8.3.

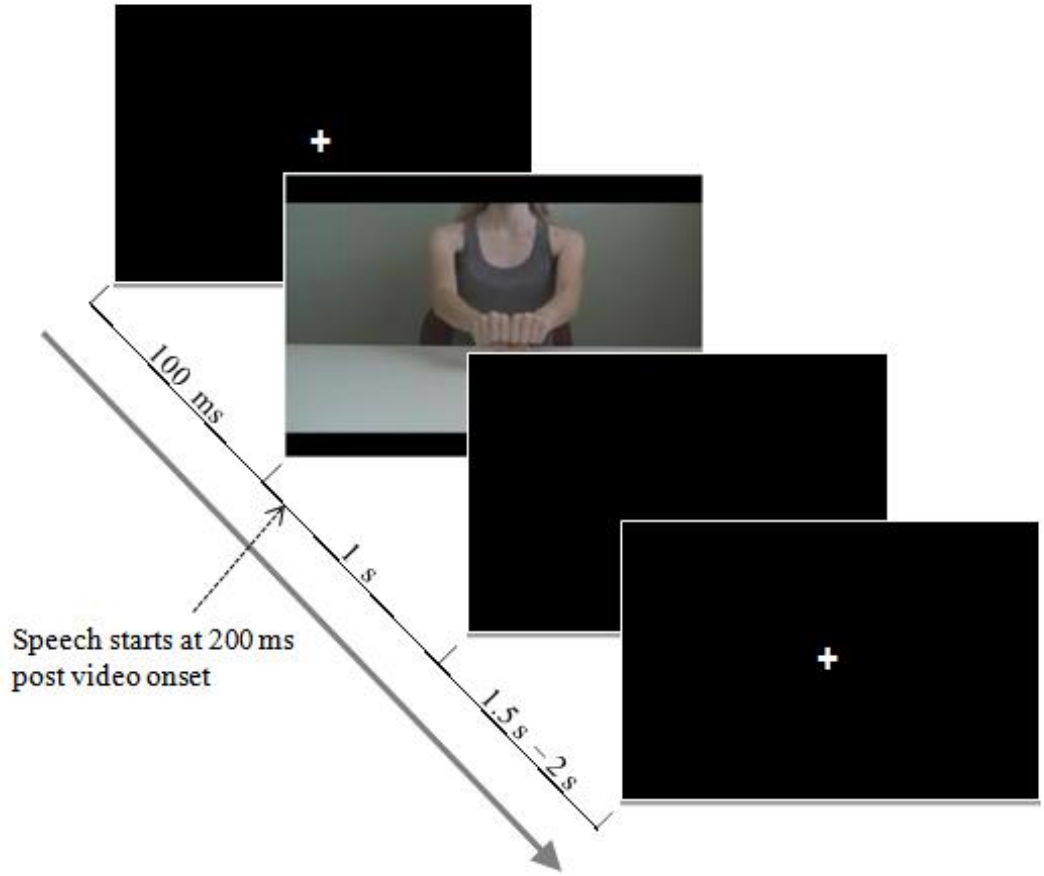

Figure 8.3. Sample gesture presentation stimuli showing display times of fixation cross (100ms) and video clip (1 s) and variable ISI (1.5 - 2 s). Speech starts at 200 ms into video clip.

The list of words, the timing of the stimulus presentation, and the framing of the clips closely resembles the stimulus outlined in the methodology used by Kelly et al. (2010). The clips in the presentation either contained a gesture and spoken word that were congruent with each other (e.g., a gestured motion of scissors, while speaking the word 'cut') or a gesture and spoken word that were incongruent with one another (e.g., gesturing a mimed screwdriver action with wrist rotation, while speaking the word 'close'). All except two of the stimulus words (omitted due to a programming error) were presented in both congruent and incongruent iterations ultimately leading to the presentation of 26 congruent and 24 incongruent video

clips. Each of the pairings is shown in Table 8.1. To check the suitability of the congruency manipulation, a group of individuals (n = 6) assessed the stimulus and unanimously agreed (on a dichotomous yes/no scale) that each of the congruent gestures matched the spoken word, and that the incongruent gestures did not match the paired spoken words.

Table 8.1.

Stimulus Word and Gesture Pairings

Spoken Word	Incongruent Gesture Pairing
Break	*No incongruent version*
Brush	Shake
Chop	Mow
Close	Screw
Cut	Stir
Hammer	Twist
Knock	Write
Lift	Stab
Mow	Turn
Punch	Wipe
Roll	Hammer
Saw	Type
Screw	Cut
Scrub	*No incongruent version*
Shake	Close
Squeeze	Lift
Stab	Sweep
Stir	Break
Sweep	Tear
Tear	Roll
Turn	Chop
Twist	Knock
Type	Brush

Wipe	Wring
Wring	Scrub
Write	Saw

Both incongruent and congruent video clips were presented in a randomised order within a single test block. Upon viewing each clip, participants were required to respond as quickly as possible as to whether the spoken word matched the gesture shown. Participants responded by pressing one of two buttons on a hand-held control device. A large-print instruction sheet was present throughout testing to ensure participants were familiar with which button corresponded 'yes' response and which corresponded to a 'no' response.

Sentence Task

In the Sentence Task, participants were presented with 48 simple, six and seven word sentences, which were presented on a computer screen one word at a time. Half of the sentences concluded with a word that was semantically congruent with preceding words and the other half concluded with words incongruent with the preceding words. The full list of sentence stimuli used can be seen in Appendix F and some example sentences can be seen below in Table 8.2.

Table 8.2.

Sample Sentences with Alternate Terminal Words.

	Terminal word	
Sentence	Congruent	Incongruent
The colour of grass is	green.	slow.
She’s not a boy, she’s a	girl.	work.

The sentence stimuli as well as the presentation protocol used are based largely on those used by Pratt, Kemp, and Martin (1996) and Sander (2008), who examined ERP components while participants read sentences concluding with incongruent semantic information. During sentence presentation each word (including appropriate capitalisation and punctuation) was shown on screen in black 48 point Times New Roman font on a light grey background. Each individual word appeared on screen for a duration of 500 ms with an ISI of 500 ms between words. The final word was followed by a 500 ms ISI and then 'XXXX' was displayed on screen for a waiting period of 1500 ms. An ISI of 250 ms followed the waiting period, then a '?' was displayed on screen and participants were required to respond (within a 2 s time-limit). Similar to the Gesture Task, participants pressed a button on a hand-held controller to indicate whether or not the sentence made sense. See Figure 8.4 for graphical representation of the presentation sequence.

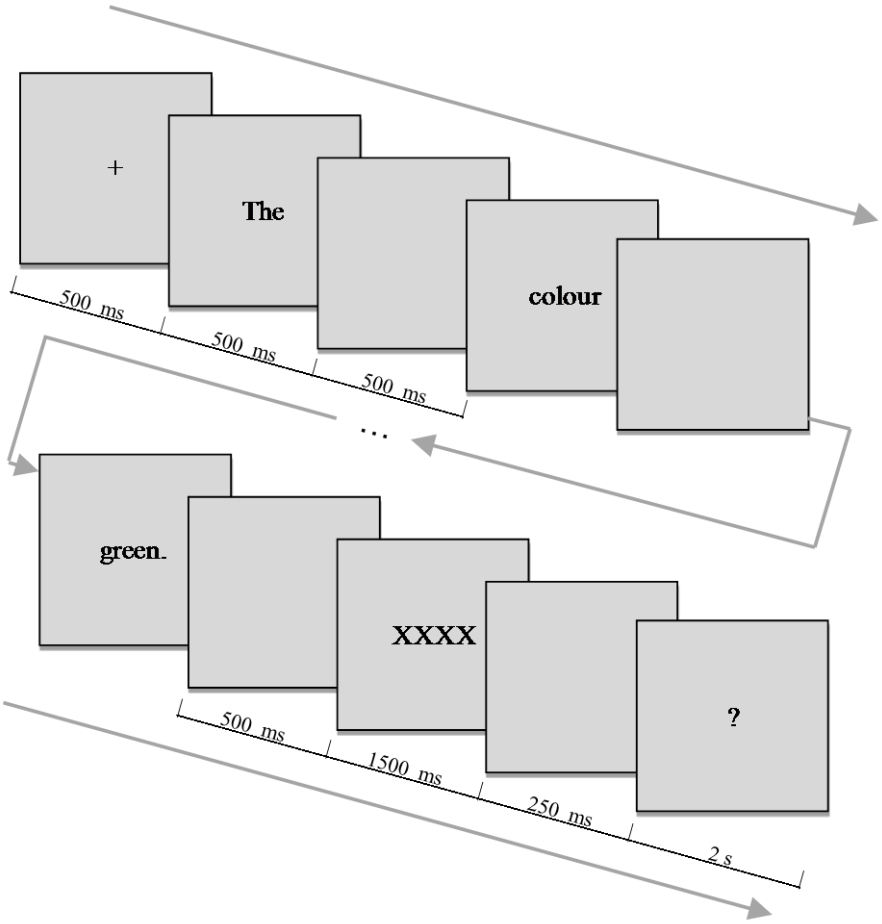

Figure 8.4. Sample of sentence stimuli presentation showing an abbreviated sequence with display periods for each frame type.

TMS and EMG Recording Apparatus

Single-pulse TMS was applied using a Magstim 200 (Magstim Co., Whitland UK) stimulator through a figure-of-eight coil (70 mm diameter of each wing). cTBS was conducted using a Magstim SuperRapid. EMG data were recorded from the first dorsal interosseous (FDI) muscle of the right hand. Ag/AgCl electrodes were attached at three sites –over the belly of the right hand FDI muscle, on the metacarpophalangeal joint of the right index finger, and a reference electrode on the styloid process of the right radius. EMG activity was band-pass filtered (20 to 1000 Hz) and amplified (X 1,000) using CED1902 amplifiers (Cambridge Electronics Design, Cambridge, UK) and then sampled using a CED Power1401 data acquisition

system. Sweeps were collected 100 ms before to 1000ms after TMS pulse was triggered using Signal 3.08 software (Cambridge Electronics Design, Cambridge, UK).

EEG Recording and Stimulus Presentation Apparatus

For EEG recording a 32 channel ANTneuro system was used with the Advanced Source Analysis (ASA v4.7, ANTneuro, Enschede, Netherlands) software to record EEG as well as participant RT. Participants were fitted with ANT WaveGuard electrode cap, which contains Ag/AgCl sintered electrodes for EEG data collection. Each cap held 32 electrodes including fixed reference electrodes at the mastoid sites, and a fixed ground frontal electrode. Free, adhesive Ag/AgCl sticky electrodes were used to record muscle activity from around the eyes. Multiple cap sizes were available to ensure that the best fitting cap was chosen for each participant. A Windows PC running EEVOKE software (ANTneuro) was used to present speech-gesture and sentence stimuli to participants. With the addition of a handheld controller, stimulus event codes and participant response codes were written directly to the to the online continuous EEG recording file. A pair of headphones were fitted during the presentation of speech-gesture stimuli to transmit the audio stream.

Procedure

Participants attended three sessions (a screening session and two test session) in the Psychology Research Centre laboratory at the University of Tasmania. Similar to the recruitment process for Study Two, the purpose of the screening session was to screen the participant pool for those who demonstrated a change in motor cortex excitability following the application of cTBS. As the aim of this study was to

investigate the role that the motor cortex may have in integrating semantic information between speech and gesture, it was important that only participants who showed an cTBS-induced reduction in motor cortex excitability were included in the testing sessions of this study. A more detailed explanation of the screening session and the selection of suitable participants in outlined in the procedure section below.

Participants were provided with a consent form, information sheet and medical questionnaire. Prior to attaching electrodes to the hands, participants were requested to wash their right hand with soapy water, and a preparation gel was used to lightly abrade the area of skin over the electrode sites. Alcohol wipes were then used to remove any dead skin cells from these areas and the electrodes were then attached. Participants were seated comfortably in an adjustable chair with their arm resting comfortably in their lap. Electrodes were connected to the amplifier and recording computer, and their outputs were assessed to ensure they were accurately recording muscle activity. If necessary, electrodes were adjusted to improve the recording signal, and participants were encouraged to relax their muscles to minimise involuntary EMG activity.

TMS Procedure

Before beginning determination of the ideal TMS location ("hotspot") and resting motor threshold (RMT) calibration, participants were fitted with an electrode cap over their head that would be used for EEG data collection in subsequent sessions. Caps were fitted during the screening session to ensure that RMT intensity that was chosen accounted for the small gap (no more than 1cm) between the scalp and the coil created by the presence of the EEG electrode cap. Similar to previous

experiments in this research project, hotspot was determined by discharging the coil over the motor cortex of the left hemisphere (approximately 5 cm lateral and 1 cm anterior to the midpoint of the head). The coil was then repeatedly discharged as the position and orientation of the coil was adjusted in order to achieve the location that resulted in maximal reliable MEP amplitudes in the right hand. Once hotspot was located, a marker sticker was attached to the cap to mark coil position. Maintaining the coil over the hotspot, RMT was then determined (defined as the minimum charge intensity needed to evoke at least 3 MEPs $\geq 50\mu$V on 5 consecutive trials).

In the screening session, following hotspot and RMT calibration to baseline motor cortex excitability (prior to cTBS), recruitment curves were obtained using intensities of 110%, 120%, 130%, 140% and 150% of RMT. Ten MEPs were elicited at each intensity. Recruitment curves were also obtained immediately following cTBS (0 minutes) and again at 5 minutes, 10 minutes, 15 minutes, 20 minutes, and 25 minutes post cTBS. The mean MEP amplitude change (as a percentage of baseline) across selected participants ('responders') was calculated and collapsed across stimulation intensities is shown in Figure 8.5. While screening the participant pool for 'responders' identifies individuals who respond to cTBS, it does not eliminate the possibility of variability in response between sessions (as demonstrated by Vernet et al., in press). Potential intraindividual variability will be elaborated upon in the discussion section.

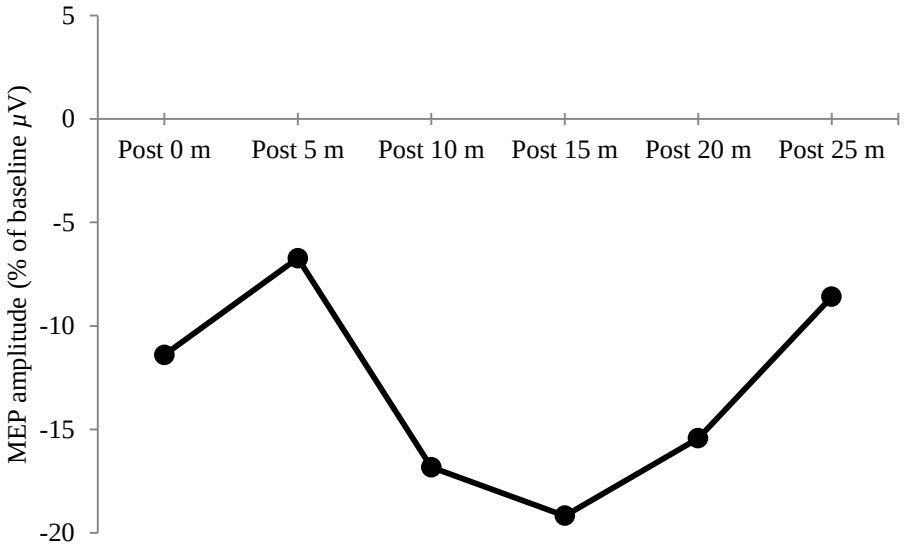

Figure 8.5. Percentage reduction in excitability for cTBS responders at all post cTBS time points. All responders showed reduction in MEP amplitude post-cTBS.

As with the previous experiment (Chapter 7: Study Two), the cTBS protocol consisted of bursts of three pulses (at a rate of 50 Hz) delivered at 200 ms intervals at 80% of active motor threshold (AMT) over a period of 20 seconds (total of 300 pulses). It was expected that this would create a reliable depression in motor cortex excitability for approximately 20 minutes (Huang et al. 2005). To determine AMT participants were asked to perform a mild voluntary contraction of FDI by pinching their thumb and forefinger together to elicit EMG activity approximately 50 μV peak-to-peak. Participants were able to see a visual trace of their motor activity (and the goal level of activity) on a computer monitor and could adjust their muscle tension accordingly. Single pulse TMS was then applied over the previously identified hotspot, and AMT was identified as the intensity at which TMS could evoke an MEP amplitude of approximately 100 μV on 3 out of 5 consecutive trials during the voluntary contraction. Following AMT determination, cTBS was then administered as per the above protocol at 80 % of AMT over the left motor cortex. In the testing sessions (sessions two and three) participants underwent a similar cTBS

setup procedure including the determination of hotspot, RMT and AMT. Unlike the screening session, the testing session setup contained no baseline/ongoing checks of motor cortex excitability. Due to the time-limited excitability effects of cTBS, it would be impractical to attempt to check excitability throughout experiemntal data collection in testing sessions. All selected participants also underwent a sham cTBS protocol in which the TMS coil was perched perpendicular to the scalp so that the coil discharged away from the cortex to ensure no magnetic stimulation occurred.

EEG Procedure

In sessions two and three, participants were fitted with an electrode cap. Prior to cap fitting, areas of bare skin that were to come in contact with electrodes were prepared to maximise conductivity. An abrasive gel was lightly applied to the skin areas across the top of the forehead, above and below the left eye, the outer canthi of both eyes, and the skin area over the mastoids. These areas were then wiped with an alcohol swab to remove debris such as makeup and dead skin cells. Following preparation, the following recording electrode sites were filled conductive gel. The sites chosen (Fp1, Fpz, Fp2, F7, F3, Fz, F4, F8, FC5, FC1, FC2, FC6, T7, C3, Cz, C4, T8, CP5, CP1, CP2, CP6, P7, P3, Pz, P4, P8, POz, O1, Oz, O2) were in accordance with the international 10-20 system of electrode placement (Jasper, 1958). All electrode sites were referenced to left and right mastoids. Horizontal and vertical electro-ocularographic (EOG) activity was recorded from the outer canthi of both eyes and above and below the left eye. Electrode impedance was kept below 10 KΩ. EEG activity was sampled continuously at 1000 Hz, and band-pass filtered (direct current - 400 Hz).

Testing Procedure

Once the electrodes cap had been properly fitted, the experimenter then conducted TMS hotspot detection, and RMT and AMT calibration as per the screening session. Following calibration, either cTBS or a sham-cTBS (control) protocol were administered. Participants were then seated in front of the testing computer and given instructions on the task they were to undertake. For the gesture task participants were told they would see words and gestures presented simultaneously and that some of the words they heard would not match the gestures they saw. For each word/gesture, they were required to respond as quickly as possible pressing one of two buttons on a handheld controller, indicating whether they matched ('yes' response) or mismatched ('no' response). For the sentence task participants were told they would see simple sentences appear on the screen one word at a time and that at the conclusion of the sentence 'XXXX' would appear on the screen followed by a question mark. Participants were asked to judge whether or not the sentence made sense, and to respond as soon as the question mark appeared on screen using the a hand held controller with the same yes/no response buttons. In both tasks participants were seated comfortably in a dark, quiet room, with their face approximately 50 cm from a computer screen. Headphones that presented speech stimuli were fitted for administration of the gesture task. The sentence task was divided into two blocks of approximately six minutes each and the gesture task was contained entirely within one five minute block. The order of gesture items and sentence items were randomised within each block and block order was counterbalanced across participants to control for the dissipation of cTBS effects on excitability. Session order (cTBS or sham administration) was also counterbalanced across participants.

Following the second session participants were contacted to arrange a third session at the closest convenience within 7 - 14 days (M = 7.5 SD = 1.07 days). Hotspot, RMT, and AMT were also determined independently for each session.

Design

For EEG data, this research employed a 2 x 2 x 2 x 2 x 3repeated measures design with 2 TMS [cTBS, Control] x 2 Task [Gesture, Sentence], x 2 Congruency [Congruent, Incongruent] x 2 Coronal location [Left, Right] x 3 Sagittal Location [Central, Centroparietal, Parietal] as the within subjects factors. The dependent variables were N400 amplitude (in μV) and latency (in ms). Regarding behavioural data, the response tasks for the sentence and gesture conditions are inherently different, as one required a rapid response (gesture) and the other a delayed response (sentence). Behavioural data for gesture and sentence stimuli can be viewed as distinct dependent variables and were therefore analysed separately. Each stimuli type was analysed using a 2 TMS [cTBS, Control] x 2 Congruency [Congruent, Incongruent] repeated measures designs, for which the dependent variable was RT in ms.

Data Analysis

EEG recordings were prepared offline in order to extract ERP data. Continuous data files were band pass filtered with 0.1 Hz high pass and 30 Hz low pass filters and then ocular artefact rejection was conducted. Continuous data were then scanned for anomalous artefacts and activity outside the range of 75 μV and 75 μV were marked as artefacts and rejected from the data set. ERP data for correct responses were epoched from 1000 ms time period commencing 100 ms before

stimulus onset and all were baseline corrected within each continuous file. For the sentence data, ERPs were epoched around the presentation of the congruent/incongruent terminal word of a sentence and were baselined to the 100 ms period preceding the presentation of the terminal word. For Gesture data, ERPs and N400 time windows were epoched around the start of the video clip, however the epoch time scale was moved so that 0 ms (the start of the ERP) coincided with the onset of the speech component of the video clips. Speech onset was at exactly 200ms into the video recording (as per Kelly et al., 2010). Kelly et al. baselined their ERP waveforms to the period of time at the start of the video clips, when the gesture had already started, with the aim of baselining waveform amplitude to account for the presence of gestural information so that the subsequent waveform could be attributed to the integration of speech information alone. In the current analysis, ERPs were instead baselined to the 100 ms period preceding the start of the clip, and therefore may represent the combined integration of speech and gesture information. Data from all participants yielded a minimum of 20 acceptable sweeps for each condition. Accepted sweeps were compiled into averaged waveforms at each site for each participant in each condition. ERP data for the N400 peak were calculated in terms of Peak Amplitude and Peak Latency within the predetermined time window of 250 to 550 ms post stimulus presentation (Kelly et al., 2004; Kelly et al., 2010). An ASA peak detection script examined this time window for each averaged waveform and marked the most negative point as the N400 peak. The amplitude and latency data of this point were identified scored and used for further analyses.

Results

Behavioural Data

Participant response events were tagged in the continuous EEG file and therefore RT was calculated within ASA as the time difference between a stimulus and the response. The average RT can be seen in Table 8.3. As the stimulus presentation and response protocol differed greatly between Sentence and Gesture tasks it would be redundant to directly compare RT between these two tasks. For the Gesture task, RT was calculated from the start of the video clip, and for the Sentence task RT was calculated from the onset of the '?' appearing on screen. Therefore, in the Gesture task, RT is likely reflective of an amalgam of decision making, stimulus processing and motor speed. In the Sentence task, RT is likely a reflection of response time to the '?' appearing on screen, as the decision about whether or not the sentence made sense would likely have already been made in the seconds between the presentation of the last word of the sentence and the '?' response prompt. Despite this, as can be seen in Table 8.3. the RT to incongruent stimuli appear universally slower in both Gesture and Sentence tasks, however there was little difference between cTBS and Control condition.

Table 8.3.

Means and Standard Deviations (in parentheses) for RT in ms for Gesture and Sentence Tasks for Each Level of TMS and Congruency.

	Gesture		Sentence	
	Congruent	Incongruent	Congruent	Incongruent
cTBS	1065.40 (250.56)	1114.92 (234.81)	366.95 (63.49)	406.68 (89.84)
Control	1071.12 (293.82)	1098.99 (272.45)	363.44 (100.51)	394.67 (135.65)

Mean RT data were analysed in separate 2 [TMS] x 2 [Congruency] ANOVAs for Gesture and Sentence tasks. An ANOVA for Gesture task data revealed no significant main effect of TMS, $F(1,9) = .003$, $p = .96$, $g = 0.02$. There was a significant main effect of Congruency, $F(1,9) = 7.31$, $p = .02$, $g = 0.17$, as RT was faster to Congruent speech-gesture pairings ($M = 1068.26$, 95% CI [914.85, 1221.67]) than to Incongruent pairings ($M = 1106.95$, 95% CI [949.91, 1264.00]), however the effect size was small. There was also no significant interaction between TMS and Congruency conditions, $F(1,9) = .495$, $p = .50$. An ANOVA for Sentence task data also revealed no significant main effect of TMS, $F(1,9) = .058$, $p = .82$, $g = 0.08$. There was again a main effect of Congruency, $F(1,9) = 6.43$, $p = .03$, $g = 0.42$, as RT was faster following Congruent sentences ($M = 365.20$, 95% CI [314.20, 416.19]) than following Incongruent sentences ($M = 400.68$, 95% CI [331.16, 470.19]), with a small to moderate effect size. There was also no significant interaction between TMS and Congruency conditions for Sentence Task data, $F(1,9) = .149$, $p = .71$.

ERP Data

Grand average data across participants for each site is shown in Figures 8.6 and 8.7. ERP waveforms in these figures have been smoothed in MATLAB using a moving average function. This was done for visualisation purposes but not for statistical analysis. It is important to note that the upper and lower limit for the timescale differs between ERP waveforms for Gesture and Sentence data (due to epoching differences).

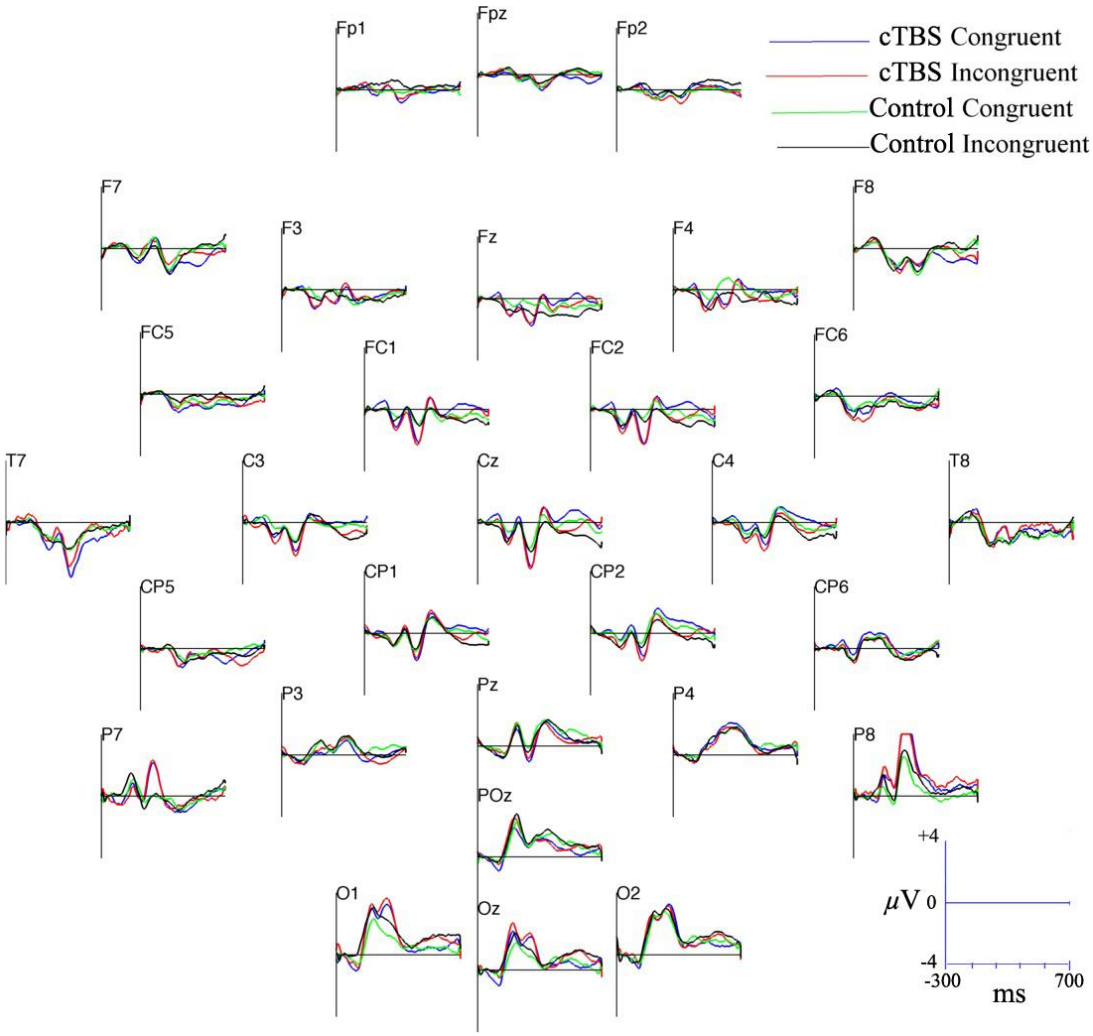

Figure 8.6 ERP grand averages for Gesture data for each level of TMS and Congruency at all recorded sites. Sites are arranged topographically and ERP waveforms have been smoothed for visual presentation.

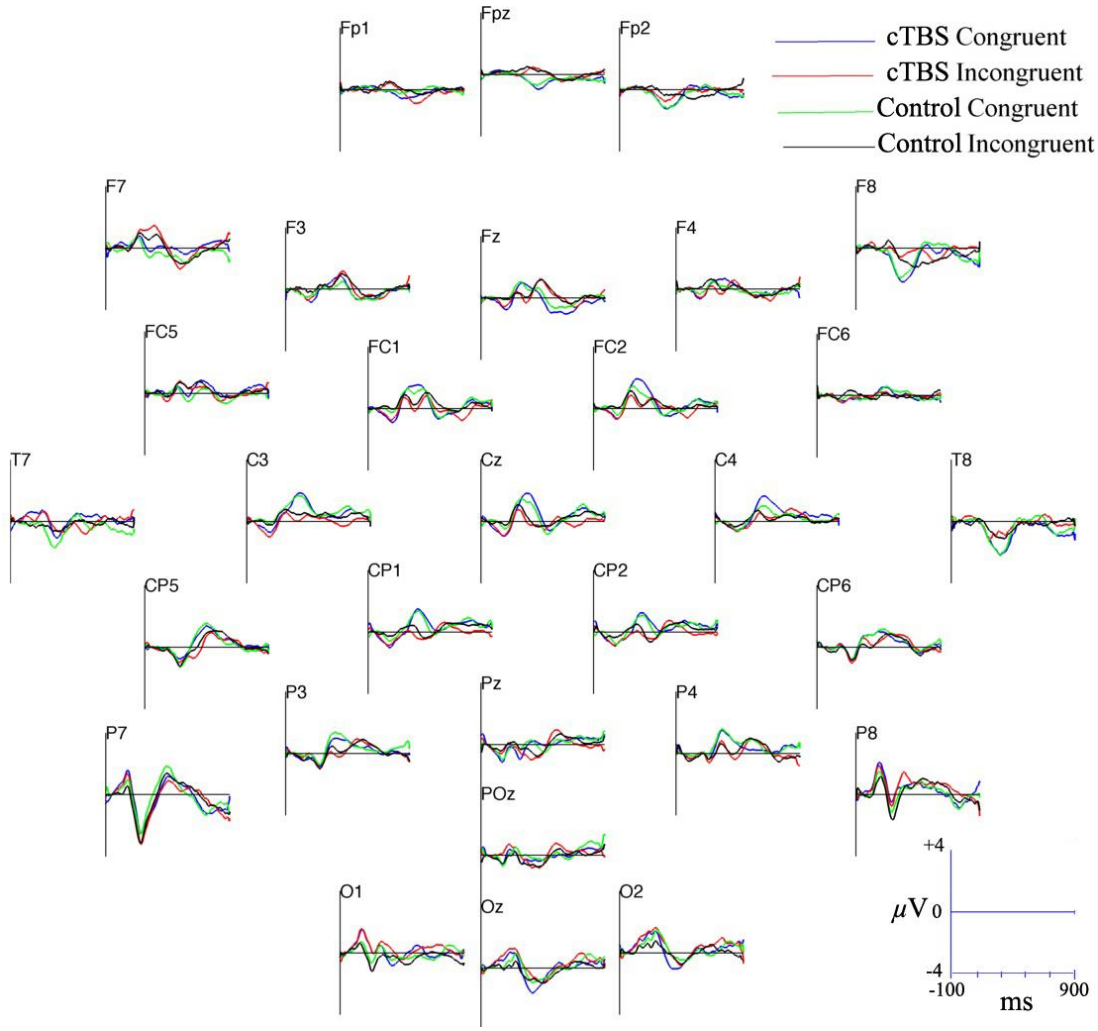

Figure 8.7. ERP grand averages for Sentence data for each level of TMS and Congruency at all recorded sites. Sites are arranged topographically and ERP waveforms have been smoothed for visual presentation.

Previous researchers have indicated that congruency N400 effects in gesture studies are most marked at central and parietal sites (e.g., Kelly et al., 2010), and at centroparietally for visual word studies (Holcomb et al. 1992; Kutas & Hillyard, 1982). Furthermore, due to specific interest in hemispheric effects of cTBS, midline sites were omitted from the analysed data. Accordingly, the following sites and groupings were selected for analysis. Sagittal groups were Central (C3 and C4), Centroparietal (CP5 and CP6) and Parietal (P3 and P4). Coronal groups were Left (C3, CP5 and P3), and Right (C4, CP6, and P4). Figures have been provided to show the ERP waveforms averaged within Left and Right sites for both Gesture (Figure

8.8) and Sentence data (Figure 8.9). These figures have been provided to show examples of averaged waveform at higher resolution. These data have been smoothed in MATLAB, are collapsed across cTBS/Control conditions, and averaged across multiple sites for visualisation purposes and were not used for analysis. Similar to grand averaging data, it is important to note that the time scale limits vary between Gesture and Sentence waveforms. A separate analysis including midline sites, but excluding centroparietal (due to the lack of midline CPz recording), examining Central and Parietal Left, Midline, and Right cites has been included as an appendix (Appendix G).

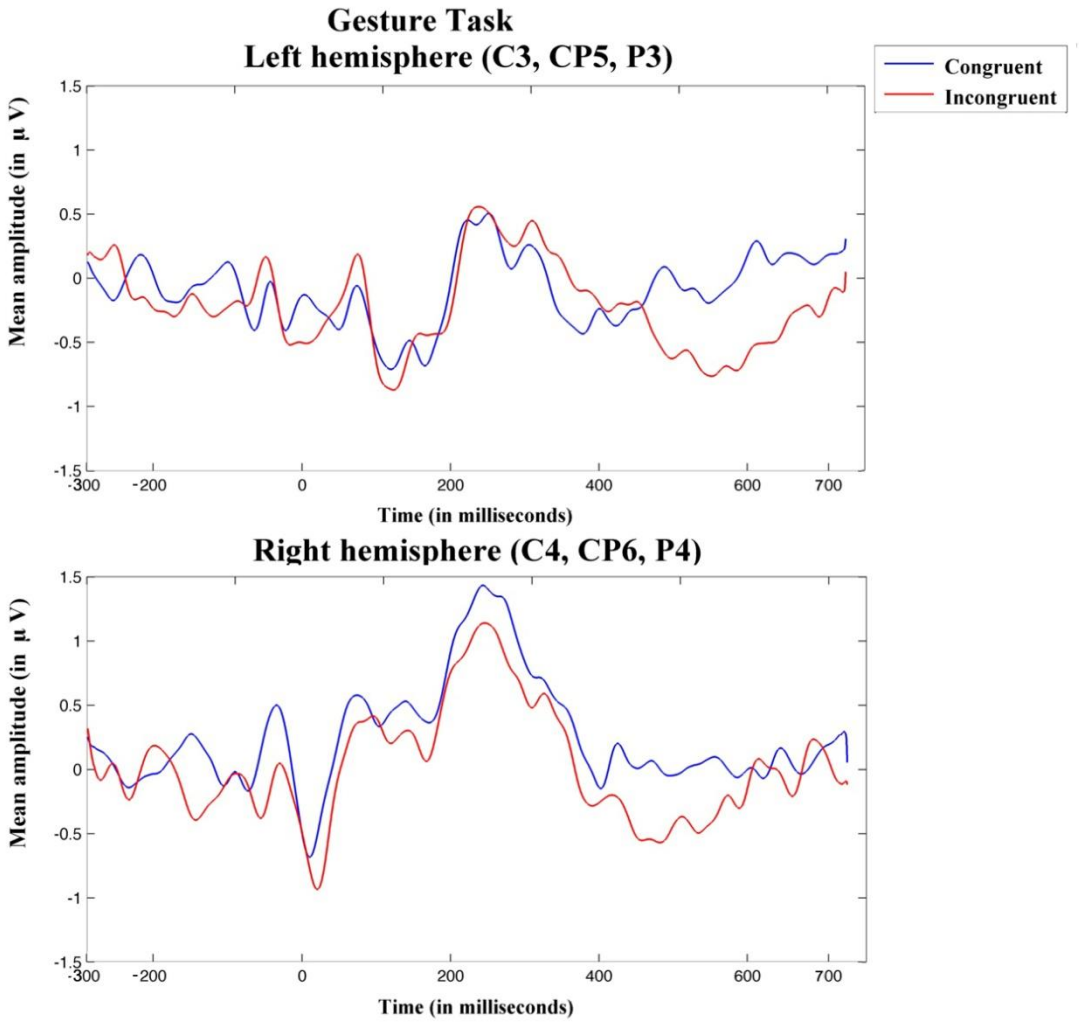

Figure 8.8. Gesture data ERP waveforms collapsed across cTBS/Control conditions and averaged across three sites for the Left and Right hemispheres. N400 peak detection window was between 250 and 550 ms post speech-onset (0 ms), and the N400 for incongruent stimuli appears more negative than congruent stimuli in both hemispheres.

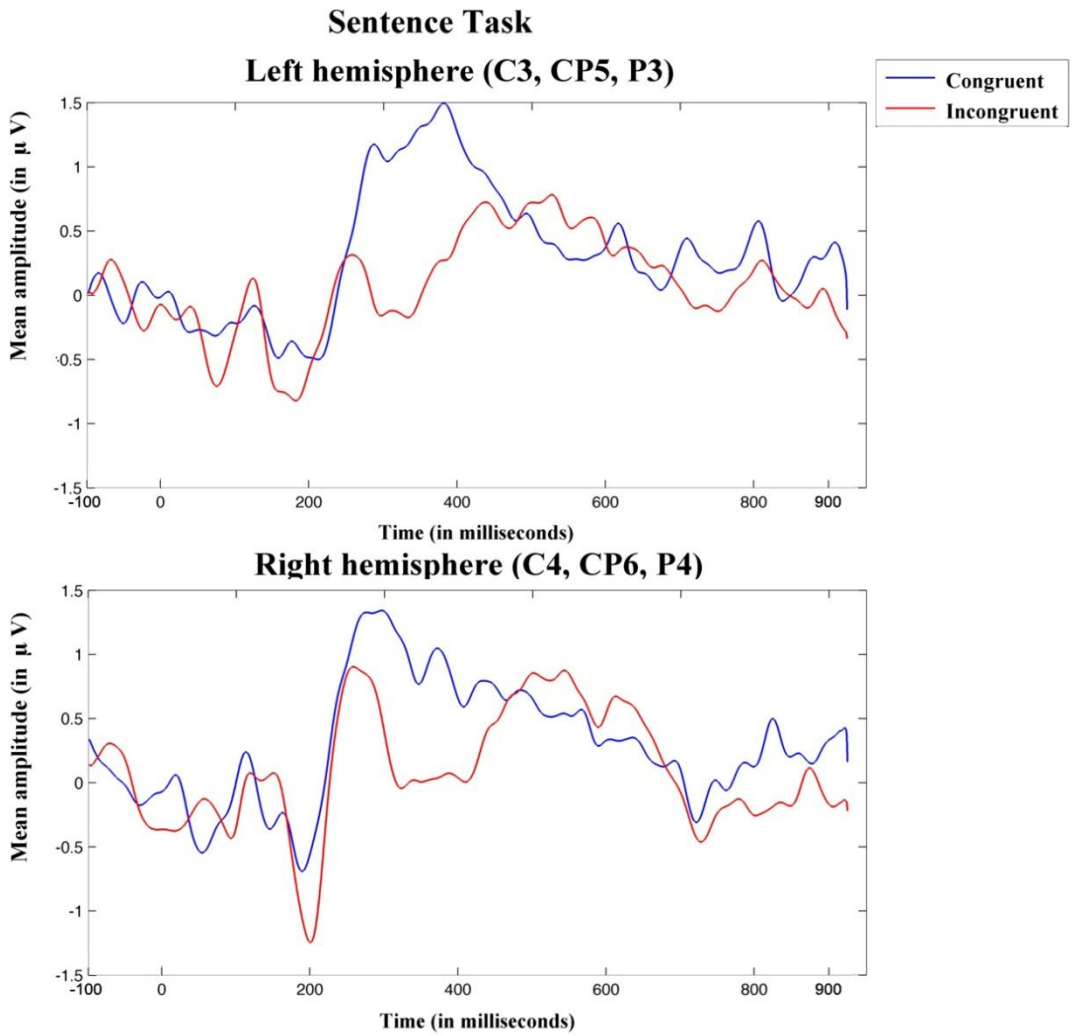

Figure 8.9. Sentence data ERP waveforms across cTBS/Control conditions and averaged across three sites for the Left and Right hemispheres. N400 peak detection window was between 250 and 550 ms, and the N400 for incongruent stimuli appears more negative than congruent stimuli in both hemispheres.

N400 Peak Amplitude

N400 peak amplitude data were averaged across participants for each level of each condition. Average peak amplitude for the cTBS and Control conditions are shown in Tables 8.4 and 8.5. A 2 [TMS] x 2 [Task] x 2 [Congruency] x 2 [Coronal] x 3 [Sagittal] repeated measures ANOVA was conducted on peak amplitude averages.

Where necessary, Huynh-Feldt corrections were applied to account for violations of sphericity. The results of this analysis are shown in Table 8.6.

Table 8.4.

N400 Peak Amplitude Means and Standard Deviations (in parentheses) for cTBS Condition shown in μV.

		cTBS Condition					
Task	Congruency	Left Hemisphere Sites			Right Hemisphere Sites		
		C3	CP5	P3	C4	CP6	P4
Gesture	Congruent	-1.29	-2.41	-1.41	-1.43	-1.76	-0.72
		(0.92)	(1.50)	(1.31)	(1.24)	(0.56)	(1.27)
	Incongruent	-2.50	-2.39	-1.75	-2.13	-2.01	-1.03
		(1.59)	(0.62)	(0.72)	(0.80)	(0.48)	(0.99)
Sentence	Congruent	-0.71	-1.13	-0.94	-0.67	-0.98	-1.06
		(0.60)	(0.91)	(0.70)	(0.66)	(1.11)	(1.01)
	Incongruent	-1.40	-1.96	-1.52	-1.27	-1.30	-1.71
		(1.14)	(0.88)	(1.49)	(1.24)	(1.04)	(1.35)

Table 8.5.

N400 Peak Amplitude Means and Standard Deviations (in parentheses) for Control Condition shown in μV.

		Control Condition					
Task	Congruency	Left Hemisphere Sites			Right Hemisphere Sites		
		C3	CP5	P3	C4	CP6	P4
Gesture	Congruent	-1.49	-1.97	-1.08	-1.58	-1.77	-1.00
		(0.54)	(0.61)	(0.81)	(1.18)	(0.51)	(1.28)
	Incongruent	-1.54	-1.92	-1.07	-2.13	-1.84	-1.05
		(1.38)	(0.70)	(0.95)	(1.23)	(1.06)	(1.11)
Sentence	Congruent	-0.83	-1.40	-0.83	-0.82	-0.99	-1.06
		(1.26)	(1.19)	(0.86)	(0.83)	(0.81)	(1.17)
	Incongruent	-1.40	-1.96	-1.52	-1.27	-1.30	-1.71
		(1.14)	(0.88)	(1.49)	(1.24)	(1.04)	(1.35)

Table 8.6.

F statistic, p-value, Degrees of Freedom, and Effect Size for Average N400 Peak Amplitude ANOVA.

Comparison	*df*	*F*	*p*	η^2_p
TMS	1,9	1.11	.32	.11
Task	1,9	9.27	.01*	.51
Congruency	1,9	15.10	.004*	.63
Sagittal	2,14	3.38	.06#	.27
Coronal	1,9	0.21	.66	.02
TMS × Task	1,9	0.92	.36	.09
TMS × Congruency	1,9	6.10	.04*	.40
Task × Congruency	1,9	1.19	.30	.12
TMS × Task × Congruency	1,9	0.18	.68	.02
TMS × Sagittal	1,12	0.33	.63	.04
Task × Sagittal	2,14	2.14	.16	.19
TMS × Task × Sagittal	2,18	0.42	.67	.04
Congruency × Sagittal	2,18	1.80	.19	.17
TMS × Congruency × Sagittal	2,15	0.64	.51	.07
Task × Congruency × Sagittal	2,15	1.17	.33	.11
TMS × Task × Congruency × Sagittal	2,16	0.09	.90	.01
TMS × Coronal	1,9	6.07	.04*	.40
Task × Coronal	1,9	0.75	.41	.08
TMS × Task × Coronal	1,9	0.35	.57	.04
Congruency × Coronal	1,9	0.15	.71	.02
TMS × Congruency × Coronal	1,9	0.72	.42	.07
Task × Congruency × Coronal	1,9	0.004	.95	<.001
TMS × Task × Congruency × Coronal	1,9	0.08	.79	.01
Sagittal × Coronal	2,18	4.81	.02*	.35
TMS × Sagittal × Coronal	2,14	0.25	.73	.03
Task × Sagittal × Coronal	2,18	1.91	.18	.18
TMS × Task × Sagittal × Coronal	2,18	0.08	.93	.01
Congruency × Sagittal × Coronal	1,13	0.03	.93	.003
TMS × Congruency × Sagittal × Coronal	2,18	0.66	.53	.07
Task × Congruency × Sagittal × Coronal	2,18	0.51	.61	.05
TMS × Task × Congruency × Sagittal × Coronal	2,15	1.49	.25	.14

NB: * designates $p < .05$, and # designates $p < .08$

As shown in Table 8.6, the ANOVA revealed no significant main effect of TMS, though a small to moderate effect size, g = 0.37. However there was a significant effect of Task, with N400 amplitude being more negative for Gesture stimuli (M = -1.64, 95% CI [-1.83, -1.45]) compared Sentence stimuli (M = -1.21, 95% CI [-1.48, -0.95]). There was also a main effect of Congruency, with N400 amplitude displaying greater negativity following Incongruent stimuli (M = -1.63, 95% CI [-1.85, -1.41]) compared to Congruent stimuli (M = -1.22, 95% CI [-1.41, -1.04], g = 1.43). There was also trend towards a main effect of Sagittal location. Sidak adjusted pairwise comparisons revealed no significant difference between Central (M = -1.39, 95% CI [-1.73, -1.01]) and Centroparietal sites (M = -1.71, 95% CI [-1.90, -1.51]), but did show that at Parietal sites, N400 was less negative (M = -1.18, 95% CI [-1.51, -0.85]) than at Centroparietal sites (p = .03, g = 1.38). No main effect of Coronal location was found.

The ANOVA on amplitude data (Table 8.6) also revealed a significant interaction between TMS condition and congruency type (means shown in Figure 8.10). In line with the main effect of congruency, Sidak adjusted pairwise comparisons revealed incongruent stimuli yielded a more negative N400 peak compared to congruent stimuli regardless of the presence or absence of cTBS (p =.003, g = 1.59, and p = .019, g = 0.80, respectively). Furthermore, these comparisons also revealed that while there was no significant difference in congruent-word N400 amplitude between cTBS and control, N400 trended toward greater negativity to incongruent words following cTBS compared to control with a moderate effect size(p = .068, g = 0.67)

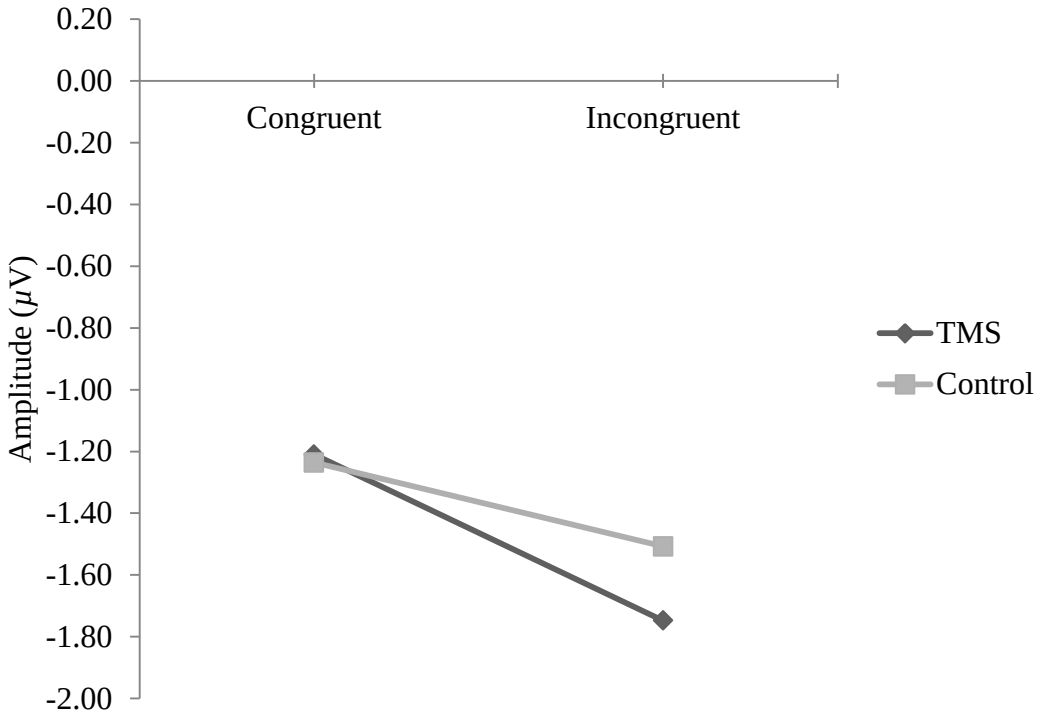

Figure 8.10. Interaction between TMS condition and Congruency. N400 amplitude is similar cTBS and Control conditions for congruent words and is globally more negative for incongruent words. Greater negativity for incongruent words is even more pronounced in the cTBS condition.

As per Table 8.6 there was also a significant interaction between TMS condition and Coronal location (shown in Figure 8.11). Sidak adjusted pairwise comparisons revealed that at Left sites N400 was significantly more negative following cTBS compared to Control with a large effect size (p = .002, g = 0.74), whereas at Right sites N400 amplitude trended towards being more negative in the Control condition compared to cTBS though with a small effect size (p = .069, g = 0.14). These results indicates that cTBS is having unique amplification effects of on the N400 peak in the left hemisphere in this sample, effects which are not seen in the right hemisphere, and are trending toward indicating the opposite effect.

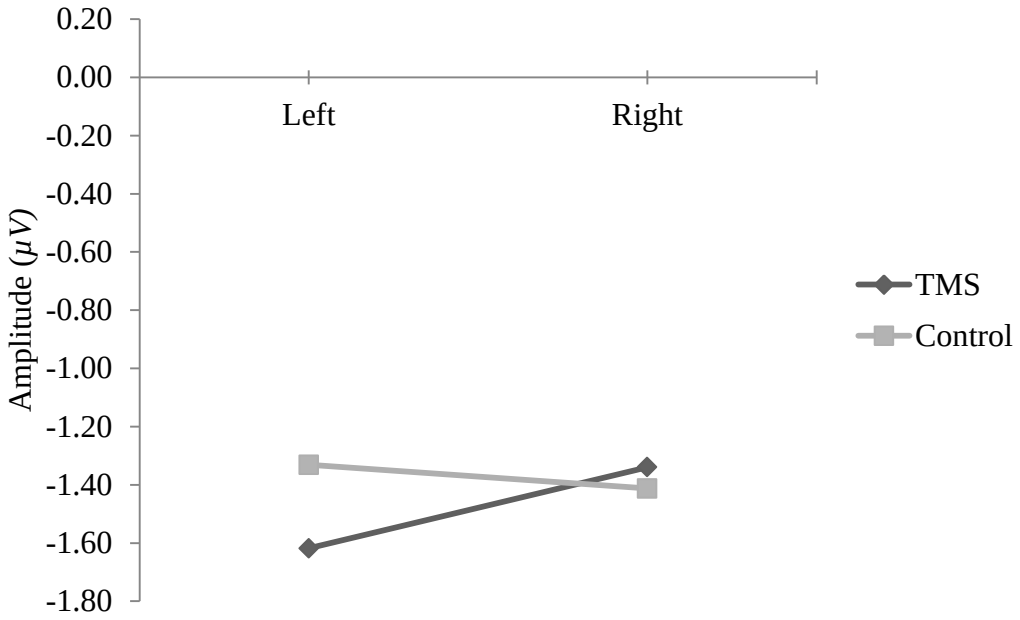

Figure 8.11. Interaction between TMS condition and Coronal location. N400 amplitude is more negative in the Left hemisphere in the cTBS condition compared to Control however there is a trend towards the opposite effect in the Right hemisphere.

The data in Table 8.6 also show a significant interaction between Sagittal and Coronal sites. Sidak adjusted pairwise comparisons revealed that at left hemisphere sites, N400 was most negative in the centroparietal region (M = -1.87, 95% CI [-2.21, -1.53]) compared to both the central (M = -1.35, 95% CI [-1.77, -0.93], p = .003, g = 0.96) and parietal sites (M = -1.20, 95% CI [-1.59, -0.82], p = .086, trend, g = 0.96). Pairwise comparisons of this interaction also revealed no significant difference between sagittal areas in the right hemisphere, nor any significant site by site differences between left and right sites. The ANOVA revealed no further significant interactions.

N400 Peak Latency

As per the peak amplitude data, N400 peak latency data were averaged across participants for each level of each condition. Average peak latency for the cTBS and

Control conditions are shown in Tables 8.7 and 8.8. A 2 [TMS] x 2 [Task] x 2 [Congruency] x 2 [Coronal] x 3 [Sagittal] repeated measures ANOVA was conducted on latency averages. Where necessary, Huynh-Feldt corrections were applied to account for violations of sphericity. The results of this analysis are shown in Table 8.9.

Table 8.7.

N400 Peak Latency Means and Standard Deviations (in parentheses) for cTBS Condition shown in ms.

		cTBS Condition					
Task	Congruency	Left Hemisphere Sites			Right Hemisphere Sites		
		C3	CP5	P3	C4	CP6	P4
Gesture	Congruent	405.08	396.68	377.34	370.80	442.97	416.80
		(92.76)	(94.45)	(73.83)	(101.76)	(41.69)	(84.83)
	Incongruent	432.03	445.21	451.37	459.77	426.27	443.55
		(111.93)	(101.17)	(107.58)	(84.02)	(82.25)	(81.43)
Sentence	Congruent	433.50	346.58	416.31	451.17	404.00	479.00
		(121.32)	(117.35)	(97.82)	(78.31)	(119.45)	(69.48)
	Incongruent	416.02	317.48	344.43	375.39	391.41	395.12
		(99.93)	(71.54)	(80.68)	(76.42)	(115.22)	(89.27)

Table 8.8.

N400 Peak Latency Means and Standard Deviations (in parentheses) for Control Condition shown in ms.

		Control Condition					
Task	Congruency	Left Hemisphere Sites			Right Hemisphere Sites		
		C3	CP5	P3	C4	CP6	P4
Gesture	Congruent	408.30	411.13	372.17	443.85	454.79	444.43
		(75.60)	(103.43)	(78.49)	(83.83)	(70.64)	(89.65)
	Incongruent	449.61	441.31	412.11	482.52	458.79	447.56
		(106.97)	(105.19)	(94.40)	(55.11)	(61.32)	(72.21)
Sentence	Congruent	398.63	381.54	474.80	432.42	401.07	377.64
		(123.58)	(135.30)	(76.19)	(70.55)	(99.44)	(90.10)
	Incongruent	-1.40	-1.96	-1.52	-1.27	-1.30	-1.71
		(1.14)	(0.88)	(1.49)	(1.24)	(1.04)	(1.35)

Table 8.9.

F statistic, p-value, Degrees of Freedom, and Effect Size for Average N400 Peak Latency ANOVA.

Comparison	*df*	*F*	*p*	η^2_p
TMS	1,9	0.01	.93	.001
Task	1,9	30.96	<.001*	.78
Congruency	1,9	0.67	.43	.07
Sagittal	2,18	0.62	.55	.07
Coronal	1,9	2.84	.13	.24
TMS × Task	1,9	3.36	.10	.27
TMS × Congruency	1,9	0.25	.63	.03
Task × Congruency	1,9	19.07	.002*	.68
TMS × Task × Congruency	1,9	0.13	.72	.02
TMS × Sagittal	2,18	0.28	.76	.03
Task × Sagittal	2,17	4.15	.04*	.32
TMS × Task × Sagittal	2,16	0.85	.43	.09
Congruency × Sagittal	2,18	1.87	.18	.17
TMS × Congruency × Sagittal	1,11	0.13	.78	.01
Task × Congruency × Sagittal	2,17	0.95	.40	.10
TMS × Task × Congruency × Sagittal	1,13	1.09	.35	.11
TMS × Coronal	1,9	0.16	.70	.02
Task × Coronal	1,9	0.09	.77	.10
TMS × Task × Coronal	1,9	15.88	.003*	.64
Congruency × Coronal	1,9	0.93	.36	.09
TMS × Congruency × Coronal	1,9	0.25	.63	.03
Task × Congruency × Coronal	1,9	0.26	.63	.03
TMS × Task × Congruency × Coronal	1,9	0.50	.50	.05
Sagittal × Coronal	2,18	0.75	.49	.08
TMS × Sagittal × Coronal	1,13	6.73	.02*	.43
Task × Sagittal × Coronal	2,16	0.81	.45	.08
TMS × Task × Sagittal × Coronal	2,18	2.16	.14	.19
Congruency × Sagittal × Coronal	2,17	0.10	.89	.01
TMS × Congruency × Sagittal × Coronal	2,15	0.53	.58	.06
Task × Congruency × Sagittal × Coronal	2,15	3.00	.09	.25
TMS × Task × Congruency × Sagittal × Coronal	2,16	0.43	.64	.05

NB: * designates $p < .05$

As can be seen in Table 8.9, there was no significant main effect of TMS condition (g = 0.04), Congruency (g = 0.31), Coronal location (despite the large effect size, g = 0.74), or Sagittal location. There was a significant main effect of Task with the N400 Peak occurring later in response to Gesture stimuli (M = 428.94, 95% CI [414.37, 443.5]) compared to Sentence stimuli (M = 391.96, 95% CI [375.44, 408.48]), with a very large effect size (g = 1.70). The main effect of Task was modified by the a significant interaction between Task and Congruency (shown in Figure 8.12). Sidak adjusted pairwise comparisons revealed Gesture stimuli elicited N400 peaks significantly later for Incongruent items compared Congruent items (p = .032, g = 1.16), whereas Sentences elicited N400 peak significantly earlier for Incongruent compared to Congruent items (p = .005, g = 1.58). Furthermore, while Gesture and Sentence stimuli elicited similar N400 latencies when the items were congruent, Sentence stimuli elicited significantly shorter N400 latencies than Gesture stimuli when the items were incongruent (p <.001, g = 2.68). The significant differences and large effect sizes produced by this analysis show very different N400 latencies, this is possibly indicative of distinct temporal processing discrepancies between printed words and gestures when these stimuli are presented incongruently.

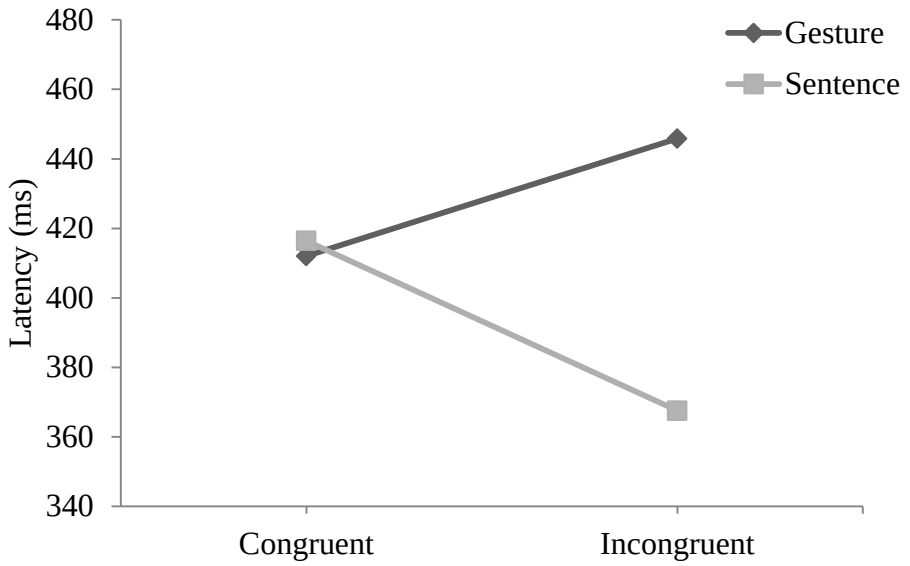

Figure 8.12. Interaction between Task and Congruency condition. N400 peak latency is similar to Gestures and Sentences when the stimuli are congruent. However peak latency is delayed when Gesture stimuli are incongruent, and is shortened when Sentence stimuli are incongruent.

The ANOVA also revealed a significant interaction between Task and Sagittal location (as per data in Table 8.9). Examining Figure 8.13, it appears that the data follow a pattern that would be predicted by the main effect of Task, as Gesture stimuli elicited later N400 peaks compared to Sentence stimuli at Centroparietal sites (p = .004, g = 1.64) and trended towards similar results at Central and Parietal sites (p = .074, g = 0.53, and p = .054, g = 0.40 respectively). This interaction seems to be largely driven by the distinct shortening of N400 latencies at centroparietal sites for sentence stimuli.

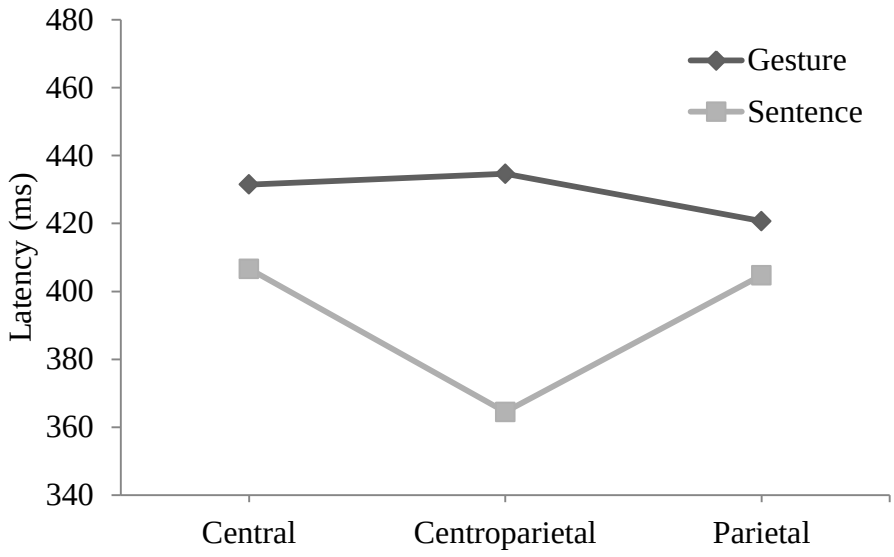

Figure 8.13. Interaction between Task and Sagittal location. Sentence stimuli elicit an earlier N400 peak latency than Gesture stimuli, particularly at Central sites.

An ANOVA of the latency data also yielded a significant TMS by Task by Coronal site interaction (see Table 8.9). Sidak adjusted pairwise comparisons revealed that in the cTBS condition, there was no significant difference between Sentence and Gesture N400 latency in the Right hemisphere sites, however there was a trend toward significance in the Left hemisphere with a large effect size (p = .062, g = 0.95) , with Gesture eliciting later N400 peaks than Sentence stimuli. This difference becomes less meaningful when looking at the left hemisphere sites in both cTBS and control conditions, as there is little difference between cTBS and control at left sites (as shown in the Figures 8.14 and 8.15). Comparatively, in the Control condition, it was found that N400 latency to Gesture and Sentence did not significantly differ in the Left hemisphere, but in fact differed in the Right hemisphere as Gesture elicited later N400 peaks than Sentence in this region (p = .001, g = 2.29). Additionally in the Control condition, N400 had a delayed latency for Gesture in the Right hemisphere compared to Left hemisphere (p = .056, trend, g = 1.16), an effect not observed in the cTBS condition.

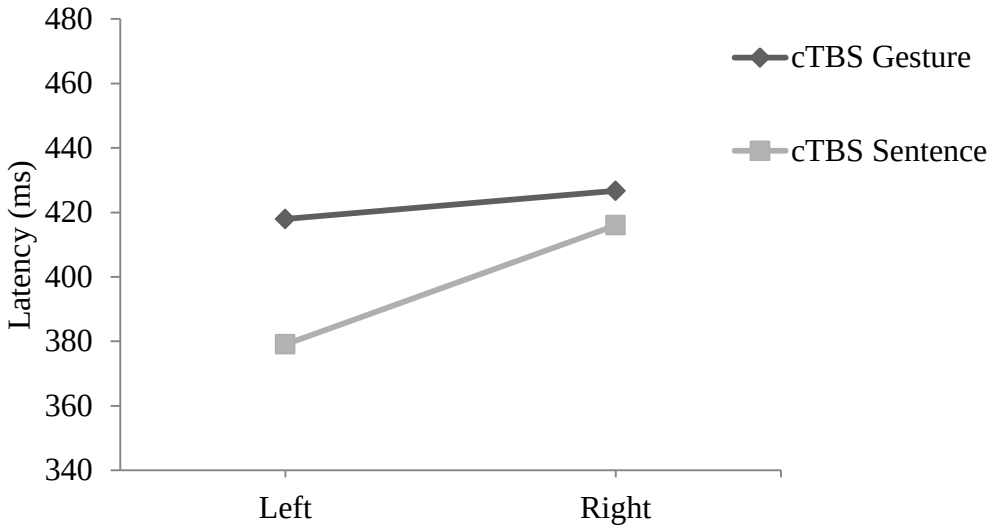

Figure 8.14. cTBS data for interaction between TMS condition Task and Coronal location. When cTBS is applied, N400 peak latency was similar between Sentence and Gesture stimuli in the right hemisphere, but the N400 peak to Sentence occurs earlier than gesture in the right hemisphere.

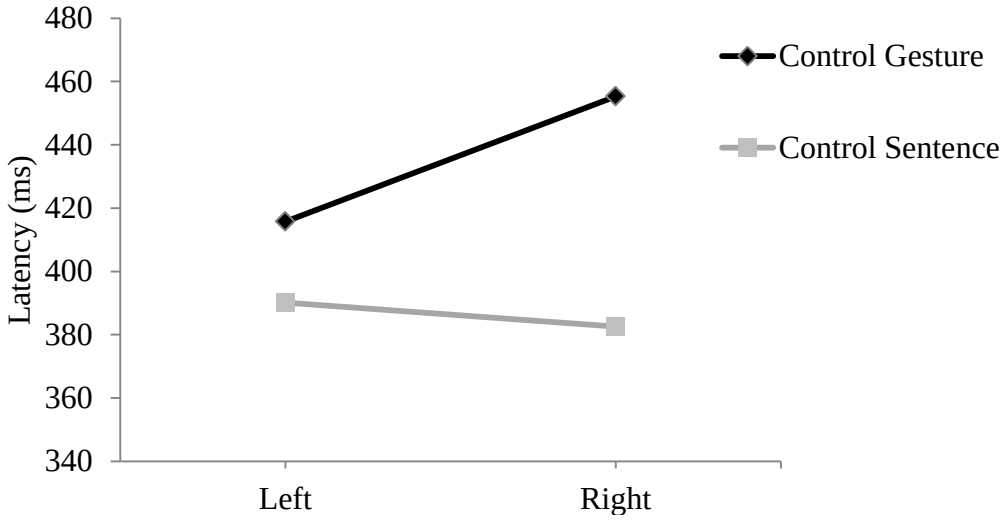

Figure 8.15. Control data for interaction between TMS condition Task and Coronal location. Contrasting with Figure 8.14, without the application of cTBS, N400 peak was similar between Sentence and Gesture stimuli in the left hemisphere, whereas in the right hemisphere, N400 peak to Sentence occurs earlier than gesture.

There was also an interaction between TMS condition, Sagittal site and Coronal site (see Table 8.9), which was largely driven by a change in activity at the

Parietal site. Despite the observation that there appeared to be differences in activity in the Central sites as well, Sidak adjusted pairwise comparisons only revealed that in the cTBS condition, Right hemisphere parietal N400 peak latencies were significantly later than Left hemisphere Parietal site latencies with a moderate effect size (p = .043, g = 0.64), and there were no other significant pairwise comparisons. The ANOVA on the latency data revealed no further significant interactions.

Discussion

The aim of this study was to investigate the impact that cTBS-induced motor cortex depression may have on the processing of gestural information, with a specific focus on investigating potential changes in the N400 ERP component in a semantic congruency paradigm. As expected, more negative N400 amplitudes were seen for incongruent compared to congruent stimuli, regardless of whether the ERPs were sampled around printed sentence endings or simultaneous speech-gesture pairings. This evidence supports the well-established previous findings indicating more negative N400 peaks for semantically incongruent printed-word stimuli (e.g., Kutas & Hillyard, 1984) and speech-gesture pairings (Kelly et al., 2010), and is indicative that the congruency manipulation worked in this particular experiment for both stimuli types.

It was expected that the congruency effect would be reflected differently in sentence and gesture stimuli as a result of cTBS depressing motor cortex activity, and specifically impacting the processing of gesture stimuli compared to sentence stimuli. It was therefore hypothesised that there would be an increase in N400 amplitude specific to the gesture stimuli that would be present following cTBS, but not in the control condition. This hypothesis (as well as the potential alternate

hypothesis of a reduction in N400 amplitude) was not supported as no gesture specific cTBS effects were found for N400 amplitude. Interestingly, there were some observed differences related to this hypothesis that are worth exploring. Firstly, it was found that overall gestures elicited more negative N400 amplitudes than printed words. Although gesture and sentence stimuli conveyed different information and the level of semantic dissonance may have been greater in one stimulus type compared with the other, it would be remiss to interpret this as evidence that gesture stimuli were perceived as being 'more incongruent' overall. As previous researchers have found that different stimuli types (e.g., auditory versus visual) communicating the same information elicit variations in N400 amplitude (Holcomb et al., 1992) it is highly possible that the overall greater negativity elicited by gestural stimuli may be attributed to inherent differences in the stimuli type, rather than indicating a between-stimuli difference in semantic congruency.

There were a further two interesting effects of cTBS seen in this data set. The first is an interaction between TMS and congruency. It was found that while incongruent stimuli led to more negative N400 amplitudes in both cTBS and control conditions (in line with the main effect of congruency), the application of cTBS created even greater negativity for incongruent words compared to control, an effect not seen in response to congruent words. All things being equal (e.g., stimulus type) more negative N400 amplitudes are generally indicative of difficulty integrating semantically dissonant information. It could therefore be suggested that reduced excitability in the motor cortex makes information integration more difficult, resulting in the larger amplitude to incongruent stimuli, regardless of the stimuli type. Before interpreting this result further, it is also important to examine the other observed effect of cTBS.

The interaction between TMS and Coronal location demonstrated distinct left hemisphere N400 amplitude effects when cTBS was applied. It is important to note that interpreting the neural source of an ERP component as being directly beneath specific site is flawed, and while all of the tasks outlined in this and previous studies evoke activity throughout cortical networks, results from combined ERP/fMRI language processing studies (such as Vitacco et al., 2002) indicate that while ERPs are not indicative of activity happening directly below the site, it would be expected that EEG data recorded from sites in specific hemisphere are representative of neural activity in that half of the brain. In the current study, it was found that N400 amplitude was more negative in the left hemisphere when cTBS was present compared to control (with a large effect size). There was also a trend toward the opposite effect in the right hemisphere, however the small effect size limits the importance of this difference. With a focus on the left hemisphere results, the observed greater N400 negativity was not specific to gestural stimuli or to incongruent words specifically, indicating that cTBS impacted N400 amplitude in both stimuli and congruency types. From these results it could possibly be suggested that reduced excitability in the hand-area of the motor cortex has led to temporary changes in the way linguistic stimuli are integrated regardless of the modality of their presentation.

Both of these results appear to indicate that the cTBS methodology applied in this particular study has led to more negative N400 amplitudes for both stimuli types: bi-hemispherically for incongruent words, with an indication that cTBS affected N400 amplitude is more negative in the left hemisphere regardless of congruency. When attempting to interpret these results it is important to be clear on the limitation of EEG and how little is known about the network-wide impacts of

cTBS in this sample. While these limitations will be explored in more detail below, briefly, while EEG data and ERP analyses provide excellent temporal fidelity, the ability to use it as a proxy for brain imaging is limited, particularly with the reduced number of sites recorded and analysed in this study. This therefore limits the ability to interpret global cortical network changes that arise as a result of cTBS over one area. Secondly, while participants were screened to check the effects of cTBS procedure on motor cortex excitability, excitability measurements could not be made during EEG data collection, and so it is impossible to tell with certainty if cTBS similarly affected excitability in the testing session. That said, it is pragmatic to speculate on what neural changes may occur following cTBS that are creating the interesting N400 amplitude effects observed in this data. Firstly it could be hypothesised that the motor cortex (specifically the hand area) is involved in processing language regardless of whether that language is motor-related or not. This explanation contrasts with previous authors who have found that when the hand area of the motor cortex is involved, it is only involved in language that is related to hands (Pulvermüller et al., 2005). It is also therefore important to note that when selecting sentence stimuli for this study, particular care was taken to ensure that the sentences contained no hand-related linguistic information..

Ultimately, to broadly claim that the hand-area of the motor cortex is involved in language processing does not fit with the body of literature, so a more detailed explanation is necessary. It is possible that the application of cTBS had broader neural effects than first expected. Previous researchers have indicated that rTMS of focal cortical areas can lead to changes in activity, excitability, and blood flow in distal regions of the brain (Denslow et al., 2005; Kobayashi et al. , 2004; Ruff et al., 2009). In an fMRI study, Denslow et al. found that rTMS over the hand-

area of the motor cortex led to detectable changes in blood flow in multiple brain regions considered to be part of the cortical motor network including other areas of the primary motor cortex as well as the premotor and supplementary motor cortices. How this links in with the results observed in the current study may relate back to the emerging understanding of the mirror neuron system. Previous researchers have found that the mirror neuron system, which incorporates motor regions such as the pre-motor cortex in non-human primates, is activated by the observation of meaningful action (Kohler et al., 2002). It is believed that in these cases the mirror neuron system activity is representative of cortical networks mimicking neuronal activity that would be required for the individual to perform the action themselves. In human research, mirror neuron activation is suspected to occur when observing action (Rizzolatti & Craighero, 2004), but is also thought to occur when perceiving speech (Wilson & Iacoboni, 2006) and when reading in both motor and non-motor related text (de Zubicaray, Postle, McMahon, Meredith, Ashton, 2010; Hauk et al., 2004) as the mirror neuron system is engaged to simulate the motor plan required to produce the language sounds it is hearing or seeing. Therefore, greater difficultly integrating semantic information (as indexed by more negative N400 amplitudes) in this study may be the result of diffuse cTBS-induced motor network changes creating global impacts on the mirror neuron system's involvement in the processing of language regardless of whether it is presented in text, or as combined speech and gesture. The fact that this effect is more notable in the left hemisphere regardless of congruency may speak to the left lateralization of linguistic functions, and is representative of the local ipsilateral impacts of cTBS. Again it is important to note that this explanation is highly speculative, particularly considering the limitations of

the variables recorded in this study as well as the rapidly developing understanding of the mirror neuron system.

Regarding analysis of N400 latency data, it was hypothesised that overall, N400 latency would not differ between gesture and sentence stimuli. This hypothesis was not entirely supported by the data, as this was only true when participants were presented with congruent stimuli. When comparing N400 latencies for these stimuli, it was found while N400 for gestures and sentences was similar in response to congruent stimuli, N400 to incongruent stimuli showed some interesting differences. Namely, N400 latencies occurred earlier for incongruent compared to congruent stimuli whereas for gestures, N400 latencies were later for incongruent compared to congruent stimuli (by approximately 80 ms). Overall this means there is a distinct delay in the N400 component when processing incongruent speech-gesture stimuli compared to printed sentence stimuli. Based on the notion that the latency of N400 component is indicative of the timing of semantic integration (Moreno & Kutas, 2005), from these results it could be suggested that the integration of incongruent speech-gesture information happens much later than it does for incongruent sentence information.

When attempting to interpret this finding, there is little evidence in the literature that would inform a theory on why gesture and speech information would be processed later than written information when incongruent. As noted earlier, not only do most researchers report on N400 amplitude outcomes, other widely cited researchers suggest that latency of the N400 component is largely unwavering (Federmeier & Laszlo, 2009). A review of the literature revealed no publish N400 study in which sentence and gesture elicited ERPs were compared within the same sample. In a recent study, Balconi and Vitaloni (in press) examined N400 in

sentences and action sequences (single-image frame sequences with a congruent/incongruent final frame), however as the EEG data were collected from separate individuals no comparison on N400 amplitude or latency between groups could be drawn. Perhaps the conclusions drawn by Moreno and Kutas (2005) regarding N400 in non-dominant language may provide a more suitable explanation. As outlined earlier, Moreno and Kutas found N400 peaks were delayed when stimuli were presented in a non-dominant language compared to dominant language stimuli, a result which the authors surmise that this may be result of the extended time it would take a participant to realise that information was incongruent when presented in a non-dominant language. It is therefore suggested that in the current study, gestural information could be viewed as akin to a 'non-dominant language', and the delayed N400 peak seen in response to incongruent gesture stimulus may be the result of the extended time taken to appreciate the semantic incongruency of the gestural component of speech-gesture pairings information. Although it might be suggested that perhaps it is specifically the auditory speech component of these stimuli that delayed N400 in this study, previous researchers have not reported consistent delays when comparing N400 peaks elicited by written and spoken stimuli in the regions analysed in this study. While Holcomb et al. (1992) reported that the N400 component to auditory stimuli was delayed compared to visual stimuli, this was only the case in temporal and frontal sites. Other authors who have found small differences between visual and auditory N400 latency have attributed these to early information that allows participants to pre-empt incongruent terminal words, such as the speaker altering speech-tone prior to the presentation of an incongruent word (Kutas et al. 1987). Interpretation of the current findings must also incorporate the overall delayed latency of gesture N400s compared to sentence N400s seen as a

main effect of task type, and reflected in the interaction of task type and sagittal location. in this interaction, moderate-to-large effect size differences were observed at all three sagittal locations, all of which indicated N400 latency was delayed for gesture stimuli compared to sentence stimuli. It must be noted that in the case of this interaction, congruency was not a factor and delayed N400 peaks were seen regardless of whether stimuli were congruent or incongruent. Nevertheless, the 'gesture as a non-dominant language' theory goes some way to explain the delayed N400 component elicited by the integration of gestural information.

It was also expected that cTBS, via depression of motor cortex activity, would specifically delay the processing and recognition of semantic congruency information in gestural stimuli. On this basis, it was hypothesised that cTBS would delay the N400 peak in the gesture condition compared to no cTBS (control),whereas sentence stimuli N400 latency would remain stable regardless of cTBS application. This hypothesis was not directly supported and the data regarding the effects of cTBS on N400 across stimuli type is much more complex than originally anticipated. The TMS by condition by coronal location interaction indicates that there are stimuli specific effects resulting from the application of cTBS, but there are distinct differences in N400 between left and right hemispheres. The results show that in the both the control and cTBS condition there is no significant difference in N400 latency to sentence stimuli between the left and right hemisphere, however there are hemispheric differences in N400 latency for gestures between cTBS and control conditions. Control data shows that gestures and sentence N400 latency is not significantly different in the left hemisphere, however N400 for gesture is delayed in the right hemisphere (by approximately 70 ms). If delayed latency is an indicator of delayed processing of the semantic integration (as per

Moreno & Kutas, 2009), this alone could be interpreted as evidence that speech gestural information is normally delayed in the right hemisphere. Interestingly, when cTBS is applied, the right hemisphere delay in processing of gestural semantic information (as indexed by N400 latency) is eliminated, as it becomes non-lateralised and similar to the processing of sentences in the right hemisphere. As cTBS was applied to the left hemisphere, it would be expected that if there were to be hemisphere-specific impacts of cTBS on the N400 component, these would most likely occur in the target (left) hemisphere If ERPs are truly indicative of activity occurring within the hemisphere of the recording site, the fact that there are fluctuations in activity in the contralateral hemisphere could be interpreted as evidence of the inter-hemispheric effects of cTBS, and support of the notion that cTBS has affected broader cortical networks than just the focal area.

Regarding behavioural data, as previously outlined the RT tasks were fundamentally different for gesture and sentence data and therefore they were analysed separately for each stimuli type. That said, there was a main effect observed for both sentence and gesture data in which RT was delayed for incongruent stimuli compared to congruent stimuli. It was expected that this would be the case for gesture stimuli, due to the participants responding as soon as they had made the congruency analysis, and the delay that would be caused by perceiving incongruent stimuli (Head & Pedoe, 1990). More surprising was the finding that this incongruency delay also occurred for the sentence stimuli, despite the fact that the decision about congruency would have been made within the 2.25 seconds prior to the '?' go-signal appearing onscreen. This unexpected result may be indicative of a generally longer RT to incongruent stimuli that has been observed in studies that employ simpler congruency RT tasks with delayed response windows. Klotz and

Neumann (1999) showed participants a simple geometric priming pattern followed by a test pattern and then question about the prime pattern (e.g., "*Was there a square in the prime pair?*"). Participants were then asked to respond to indicate whether or not the priming pattern matched/mismatched the subsequent suggestion. There were multiple variations on this methodology, but importantly one procedure required a rapid decision and response, whereas another version had no time limit and financially rewarded accuracy. Klotz and Neumann found that regardless of whether the participant goal was response time or accuracy, RT for incongruent stimuli were delayed compared to congruent stimuli. Relating this back to the current study, the previous observations of a general delay in responding to incongruent stimuli (regardless of the presence of time pressure) may help to explain delayed RT to seen in both incongruent sentence and gesture stimuli.

At first glance, it may appear that for gestures the RT results support the N400 latency data that suggests incongruent gesture stimuli are processed later than congruent stimuli leading to a delayed RT, whereas for sentence the RT data do not support the finding that N400 sentence latency data show a decreased peak latency for incongruent compared to congruent stimuli. However, aside from the fundamental task differences indicated above, it is also important to acknowledge the limits of converging behavioural and electrophysiological data in this manner. ERPs are millisecond time-locked to the onset of a stimulus and therefore provide great temporal accuracy. As with many behavioural measures, recordings of RT are subject to variability in participant effort and coordination. It would therefore not be unexpected that measures with less variability (e.g., ERPs) would detect significant changes that wouldn't be detectable in a more variable behavioural measure. Following from this, it is also interesting to note that cTBS had no significant effect

on RT for either stimulus type, despite cTBS affecting the N400. It could be suggested that this is evidence that an cTBS-induced delayed N400 latency does not necessarily equate to a delay in processing, or a more salient explanation may be that this is again is evidence that RT data may be too variable to reveal differences between conditions that are detectable through EEG.

Regarding RT, there is some suggestion in the literature that cTBS can directly impact reaction time. In a simple RT task, Huang et al. (2005) found cTBS of the motor cortex delays RT in the contralateral hand by approximately 7%. In the current study, participants consistently respond using their right hand (incongruent/'No' response) and left hand (congruent/'Yes' response). In the initial design for the study, it was intended to counterbalance this by laterally switching the response keys. Due to the limited number of participants data collection was discontinued before this was achieved. While this could potentially be viewed as a confound, as can be seen in the RT data from the current study, cTBS had no specific effect on RT to incongruent stimuli in either the sentence or gesture data despite "incongruent" responses being executed with the right hand. This is evidence that in this sample, cTBS over the left motor cortex did not delay RT in the right hand.

As alluded to above, there are a number of limitations in the methodology of this study that must be acknowledged. Firstly, the final number of participants who completed all three sessions (Screening session, cTBS, and Control) only amounted to 10 individuals. Ultimately, the screening methodology used in this study was very rigorous and while it ensured that only EEG data from cTBS responders was recorded, it did limit the final sample size. Despite 25 participants being recruited in total, the limited number of participants who passed the screening was not ideal. While these numbers are comparable to other rTMS studies recruiting small numbers

of participants (e.g., *n* = 12, Daskalakis et al. 2006; *n* = 9, Huang et al. 2005; *n* = 8, Martin et al., 2006), previous ERP researchers often record data from larger samples (e.g., *n* = 15, Kelly et al. 2004; *n* = 15, Cornejo et al, 2009; *n* = 28 Kelly et al. 2010). It might be suggested that the small sample size limits the robustness of these EEG findings, but the rigorous screening methods maximised the chances of finding cTBS specific effects. Regardless, should a larger sample size be the goal of future researchers, an estimated *n* >70 of pre-screened participants may be needed get a final cTBS responder sample size reflective of the number of participants recruited by Kelly et al. (2010). A second limitation to the robustness of the data was the number of possible successful sweeps that each ERP grand average could be based on for each participant. Maximally, there was the potential for 24 - 26 sweeps for each congruency type for gesture stimuli and 24 sweeps for each congruency type for sentence stimuli. Due to the temporary nature of cTBS-induced excitability perturbation, it was only expected that cTBS would depress motor cortex excitability for 20 to 30 minutes post stimulation. Therefore both the sentence and gesture testing blocks, each containing congruent and incongruent stimuli needed to be presented with this limited time-frame. This limitation restricted the number of possible individual trials a participant could complete before the inhibitory effects of cTBS were thought to diminish. A third limitation is the variability in response to the cTBS protocol. Not only have excitability effects been found to vary between participants (Maeda, Keenan, Tormos, Topka, Pascual-Leone, 2000), in some cases they have also been found to vary within participants between stimulation sessions (Vernet et al., in press). As mentioned previously, although the screening session methodology was employed to recruit only individuals who were responders to the effects of cTBS on motor cortex excitability, there was no practical way to regularly

check excitability through the testing sessions as regular single pulse administration would have disturbed performance in the task, or created unacceptable delays to trial progression.

A fourth limitation is the speculative nature of ERP data interpretation, specifically the neural/functional underpinnings of modulations of the N400 component. As has been noted earlier, although N400 characteristics are often cited as indexing the integration of semantic information, the exact nature of what neural function can be interpreted from this is still under consideration (see Kutas and Federmeier, 2011). The topographic accuracy of ERP data also limits the interpretability of these results. Results from combined ERP/fMRI research has shown the electrical activity recorded at the site may not be reflective of actual proximal sub-electrode neural activity (Vitacco et al., 2002), and in this current study, the number of sites that were recorded and the analysis technique used ensure that these data cannot be used to make direct inferences about the functional changes in specific areas of the brain, beyond hemispheric comparisons. A final limitation to the interpretation of data from this study is therefore the lack of accompanying neuroimaging data that would assist with understanding the activity of specific regions of the brain. Recent advances in research methodologies that combine TMS with neuroimaging techniques, such as fMRI and PET, have yielded a great amount of detail on TMS-induced changes to function throughout cortical networks. Unfortunately these techniques also have their drawbacks (such as unavoidable somatosensory and auditory stimulation), and are not as easily accessible as EEG data collection facilities (Siebner et al., 2009).

This leads to the final point, in future research in this field, the addition of neuroimaging data would be of great benefit. In this study, neuroimaging would not

only have provided a clearer illustration of task specific cortical activity, it would also have assisted to clarify the possibility that cTBS is affecting neural activity more globally in regions beyond the targeted motor cortex. Kobayashi et al. (2004) theorised that a temporary rTMS-induced 'lesion' over one area of the brain which usually dominantly performs a function leads to increased excitability in the less-dominant homologous brain area in the contralateral hemisphere. Andoh and Martinot (2008) theorise that TMS of left-hemisphere dominant language areas such as Wernicke's areas may lead to increased compensatory function of the right hemisphere homologue to Wernicke's area in the superior temporal gyrus. Not limited to just the direct homologue, other researchers have proposed an even broader 'rapid reorganisation of cortical function' (Zanto, Chadick, Satris, & Gazzaley, 2013). Zanto et al. posit that following a rTMS virtual lesion, in order for the brain to maintain functionality for affected processes, there are non-focal network-wide changes. In an fMRI study, Zanto et al. reported that when rTMS was applied over the right inferior frontal junction (IFJ) and participants were asked to perform a visual memory task, there was an increase in activity in remote regions of the brain, specifically the left lateral occipital complex (LOC). As RT and accuracy were not impacted by the application of rTMS, and the LOC activity was only observed following rTMS administration, Zanto et al. concluded that the LOC could be recruited to provide some 'compensatory functionality' to ameliorate reduced excitability in the IFJ. While it would be too great a speculative leap to suggest that in the current study there is unequivocal evidence of cTBS induced rapid functional reorganisation, or interhemispheric changes in functional dominance, the possibility that these phenomena play at least some part in these results should not be dismissed.

In summary, this study has yielded some expected results that can be clearly interpreted in light of the previous research such as the well-establish increased N400 amplitude displayed greater negativity in response to incongruent sentence endings (e.g., Kutas & Hillyard, 1980), as well as speech-gesture incongruencies (Kelly et al. 2010). Additionally, there are some new findings such as the notion of increased N400 amplitude for gestural stimuli and the delayed N400 latency for incongruent gestures compared to incongruent sentences, which can both be explained as a result of the inherent differences between the two stimuli types. This study has also revealed some very specific cTBS related effects, indicating that cTBS of the motor cortex does have specific impacts on the N400 ERP component. The results show that when cTBS is applied it has specific effects on the amplitude of the N400 peak, eliciting a more negative peak for incongruent stimuli regardless of stimulus type (gestures or sentences). Further to this, gesture stimuli N400 latency specifically is also affected by the application of cTBS, however this effect was recorded as a delay in the N400 latency in the right hemisphere only. Some of these cTBS-related differences are difficult to interpret as they defy expectations and contrast with previous findings. That said, the developing understanding of the role of the mirror neuron system within the human brain, the relationship between language and action, and the very recently emerging theories on rapid cortical functional changes borne by rTMS all inform a theoretical framework in which these results may be interpreted. In conclusion, these results demonstrate that cTBS of the motor cortex is having some impact on the specific processing of simultaneously presented speech-gesture and stimuli, however there are also more notable global (and interhemispheric) impacts of cTBS on language processing and the N400 which need further empirical exploration.

Chapter 9: General Discussion

The aim of this research paper was to investigate the relationships between action and language, with a specific focus on the role of the motor cortex in language processing. Compared to the well-established understanding of traditional linguistic cortical regions (such as Broca's and Wernicke's areas), advancements in the scientific understanding of the role of the motor cortex in linguistic processing are comparatively new. Despite stark differences in the intention, design, and methodology of all three studies cited in this paper, they all employ the application of TMS, a valuable research and therapeutic technique which allows the operator to effect changes in brain excitability in a safe and non-invasive manner. By extending on previous methodology as well combining research techniques, the aim of this paper was to offer greater insight into the links between language and action and add valuable information to this research field.

Empirical Study One

The first empirical study extended on the findings of Pulvermüller et al. (2005) and Mertens (2009), and investigated the effect of single pulse TMS delivered over the hand area of the left motor cortex with varying asynchronicity to the presentation of hand-related verbs. Previously, Pulvermüller et al. had reported that when a TMS pulse of 90% resting motor threshold (RMT) intensity was delivered over the hand area of the motor cortex 150 ms after a word was presented, participants responded more quickly to hand-related verbs compared to leg-related verbs. In 2009, Mertens hypothesised a greater intensity TMS pulse (120% RMT) delivered at the same latency may induce the opposite effect, leading to an increase in reaction time (RT). Mertens found no impact of 120% intensity pulse at 90 ms post-stimulus on RT, which inspired the design of the first empirical study in this

paper. Study one aimed to investigate RT to hand-action words following different TMS pulse intensities and latencies on RT. In this experiment participants were required to identify the validity of a word presented from a list that contained hand related action verbs, non-action verbs (control) and pseudowords. Based on the findings of previous authors (Papeo et al., 2009; Pascual-Leone et al., 1992; Pulvermüller et al., 2005), four different pulse intensities (90%, 100%, 110%, 120% of RMT) and five different pulse latencies (120 ms, 150 ms, 280 ms, 230 ms, 290 ms, 350 ms post-stimulus) were chosen. Due to many participant RTs occurring prior to or approximately at 350 ms, this latency was removed from final analysis. It was hypothesised that the intensity/latency combination used by Pulvermüller et al. (90% and 150 ms) would yield a speeding of RT for action words compared to control. It was also hypothesised that the further the pulse was from this intensity or latency, the less facilitative impact it would have on RT, perhaps even delaying RT at the most distal intensity/latency combinations. Contrary to predictions, it was found that at the 90% and 150 ms intensity/latency condition RT was not faster to hand-action words, and was actually faster to control words. Interestingly, this effect was not limited only to the 90% with 150 ms combination, but was also found at 110 % with 230 ms, 120% with 120 ms, 120% with 180 ms (although with moderate effect sizes). No combination of intensity and latency yielded faster RTs to action words compared to control.

When comparing these results with those of Pulvermüller et al. (2005), it must be noted that the significant TMS induced RT change reported in their study compared RT to arm-related words with RT to leg-related words. It is therefore possible that TMS induced RT change to arm-related words may only be significant change in RT compared to action words related to a different effector (i.e., the leg),

and not an improvement of RT compared to all words more generally. It was also found that RT trended towards being faster for the TMS group compared to the sham (control group), which, with a moderate effect size, indicated that regardless of latency or intensity manipulations, TMS may speed RT. The finding that RT was faster in the TMS group is difficult to account for, and could potentially be dismissed as a non-significant result, as merely representative of differences between the TMS and control groups. However, it is possible that is indicative that TMS speeds language processing tasks regardless of the word type, or more specifically that TMS impacts processing in this particular task – word-validity judgement.

When examining the overall effects of TMS on RT, it is important to also consider the main effect of latency. Independent of TMS manipulation, word type or intensity, there was a main effect and a quadratic contrast in the latency data that demonstrated that RT was faster when pulse was delivered at 150 ms, 180 ms, and 230 ms post-stimulus, with an indication that this was maximal at the 180 ms latency. Despite RT being faster in the TMS group overall, it is interesting that there was an effect of pulse latency on RT that was similar in both TMS and control groups. In the control group, authentic TMS was not administered, and instead a sham coil (which mimics the look and sound of a real coil) was used. When interpreting the latency main effect it could be suggested that at least some of the latency-related change in RT data cannot be attributed to the neurophysiological effects of the TMS pulse on the motor cortex, as these are not present in the control condition. This leads to the conclusion that there must be other factors influencing RT that are common to both the TMS and control conditions, one of which is the acoustic 'click' heard when the TMS coil discharges. More specifically, it appears as though RT may be speeded by the participant hearing the TMS coil discharging,

even without actual stimulation, and that this acoustic cue is ideally provided 150 ms to 230 ms post-stimulus onset to maximise RT improvement. While this doesn't provide any indication of specific motor cortex and language processing interactions, it does provide further credence to previous reports of acoustic cueing effects of TMS coil discharge (Terao et al., 2007).

Empirical Study Two

The second empirical study detailed in this thesis examined the role of the motor cortex quite differently. The aim of this study was to use rTMS to create more long-lasting temporary changes to motor cortex function to investigate whether this would impact a participants ability to identify gestures. Continuous theta-burst stimulation (cTBS), a type of rTMS was applied over the left motor cortex with the intention of reducing excitability in that area of the brain for the subsequent 20 minutes. Previous researchers have demonstrated that application of rTMS to Broca's area impacted their performance in a task in which they were required to identify a three words presented on screen as text, gesture, or speech (Gentilucci, et al., 2006). Specifically, Gentilucci et al. found that rTMS of Broca's area significantly delayed RT to speech stimuli. Based on the notion that rTMS of cortical areas responsible for specific language tasks impacts task performance, it would be expected that if the motor cortex is involved in gestural processing (Skipper et al., 2007), then rTMS of the motor cortex may impact processing of gestural information. Empirical study two was therefore designed to investigate the impacts cTBS over the motor cortex on gestural processing. In this study, participants identified five stimulus words presented multiple times in four different modalities; text, actor speaking, actor gesturing, and actor speaking and gesturing simultaneously. It was predicted that if the motor cortex was involved in gestural processing there would be specific delays

in RT when cTBS was applied compared to control in the gesture, and perhaps even the gesture and speech combined conditions. It was also expected that RT to text and speech alone would be unaffected by cTBS. Contrary to predictions, there was no statistically significant effect of cTBS on RT for any of the conditions.

Considering cTBS did not even delay RT in the gesture-only condition, it could be argued that the motor cortex does not appear to be involved in processing gestural information. That said, there are alternative explanations which may account for these results while still allowing the motor cortex to be involved in gestural processing. The first explanation relates to effectiveness of the cTBS protocol in reducing excitability to a level at which it has an effect on behavioural responses (in this case RT). Some researchers have found that while physiological measures (such as excitability) may show TMS induced effects, behavioural performance is not significantly changed (Rossi et al., 2000). While there is no empirical evidence of this in the current study, some authors attribute lack of behavioural changes to the widespread effects of focal reduced excitability (e.g., Ridding & Rothwell, 2007; Thiel et al., 2006). More specifically, it is suggested that a 'virtual' (TMS-induced) lesion in one specific cortical area cause the brain to engage additional/alternative regions to continue to perform required functions. This 'rapid cortical reorganisation' (Thiel et al., 2006) therefore allows other areas of the brain to perform compensatory functions minimising the impact of cTBS, or at least minimising interruptions in observable behavioural outcomes (such as RT). The notion of non-focal impacts of rTMS may have ramifications for other research paradigms that are based on the perception that rTMS can induce reliable and focal change to specific cortical areas. Future researchers using techniques such as cTBS, need to consider the possibility

that their results may be reflective of broader cortical changes, rather than a just reduced excitability in the targeted brain region.

A second explanation for the results in this study is related to the observation that emblematic gestures were used. Emblems are gestures that are usually culturally bound and can usually be interpreted in the absence of spoken language. Based on recent fMRI data, Andric et al. (2013) suggest that due to the inherent meaning that gestural emblems evoke, they are processed by motor *and* language systems in the brain. The reason this is important to results from the current study is that regardless of the widespread cortical impacts of rTMS (via compensation), it is quite possible that traditional language areas were already capable of rapidly processing the emblematic gestures regardless of whether TMS was applied to the motor cortex. While both of these explanations indicate that cTBS of the motor cortex does not impact processing of emblematic gestures, they still offer the possibility the cTBS may impact the processing of other gesture types, such as iconic/co-speech gestures.

Empirical Study Three

Similar to study two, the third study also investigated the use of rTMS (specifically cTBS) on the hand-area of the left motor cortex and the impacts that it had on gesture processing. However, unlike study two, this study focussed on the role of the motor-cortex in processing iconic/co-speech gestures and included EEG data to provide convergent evidence for the impacts of TMS on gestural processing. The N400, a late negative going ERP waveform peak has been found to be reliably attenuated by the integration of semantically incongruent linguistic information. Specifically, N400 amplitude is more negative when linguistic stimuli are semantically incongruent with the context they are presented in compared to when stimuli are congruent (Kelly et al., 2010; Kutas & Hillyard, 1980). Interestingly,

while N400 amplitude is susceptible to fluctuations, the latency of N400 is largely fixed, save a few exceptions such as stimuli presented to bilinguals in a second language (Moreno & Kutas, 2005). Empirical study three aimed to replicate findings by other researchers demonstrating that an N400 amplitude could be elicited to speech-gesture stimuli, and that the congruency effect that is seen in response to written language would also be seen to gesture stimuli. In addition to this, under the assumption N400 component is reflective of integration of semantic linguistic information meaning, this study investigated the impact that reduced motor cortex excitability (via cTBS) may have on the integration of gestural information, as indexed by N400. Participants viewed both speech-gesture and sentence stimuli that were presented as both matching/congruent and mismatching/incongruent test items. In one session, participants viewed these stimuli following the application of cTBS and in the other session following administration of a sham cTBS procedure (control).

Analysis of the data from study three provided some interesting results independent of the effects of cTBS. Firstly, as expected N400 amplitudes were more negative for incongruent stimuli for both the gesture and sentence stimuli. Secondly, incongruent gesture stimuli produced more negative N400 peaks at a delayed latency compared to incongruent sentence stimuli. These findings are thought to be the result of the inherent differences between the stimuli types, and may indicate that gesture-speech information is integrated later than written text is. It is possible that the delay in integrating incongruent gesture-speech information (as indexed by a later N400) may be related to previous reports of similar delays when integrating incongruent information presented in a non-dominant second language (Moreno & Kutas, 2005). It was expected that if the motor cortex was involved in gestural processing, cTBS

would impact N400 amplitude in response to gesture stimuli only. This *specific* gesture-related effect of cTBS on N400 amplitude was not found, but surprisingly, when cTBS did impact N400 amplitude, the effects were seen in response to *both* gesture and sentence stimuli. Firstly, in the cTBS condition, N400 peak amplitude was more negative for both sentence and gestural data. Changes in N400 amplitude like this are usually seen when a stimulus is more difficult to semantically integrate into an existing context. The fact that this occurred regardless of stimuli type (gesture or sentence), this finding is difficult to interpret upon reflection of the literature. Reports from previous researchers largely indicate that if the motor cortex is involved in language processing, it is only involved in processing action-related linguistic information, such as action-related words or gestures (Pulvermüller et al., 2005; Skipper et al., 2007).

Regarding N400 latency, there were observed delays in the N400 peak to gestural stimuli, however this was observed only in the right hemisphere. Again this result was unexpected, and despite the fact that a specific delay to N400 peak to gesture stimuli can be explained in terms of inherent differences between the stimuli, the fact that it was observed in the right hemisphere only, and not the focally stimulated left hemisphere indicates that TMS may be impacting cortical regions beyond the targeted motor cortex. In combination, the novel findings for both N400 amplitude and latency are difficult to interpret based solely on the information gathered in this study. It appears as if there are multiple factors at play which may be influencing cortical activity, the processing of language and therefore the observed effects. Speculatively, these results may be indicative that TMS-induced suppression of motor cortex activity is having an impact on more wide-spread cortical networks. The engagement of a compensatory neural network may be at play, but due to the

insufficient topographical fidelity offered by EEG data in this study, is it not possible to confirm these hypotheses without the provision of convergent imaging data.

Conclusions

As stated above, the aim of this thesis was to provide further insight into the role of the motor cortex in language processing. Via investigating the effects of single pulse TMS on the processing of action-related language, and the impacts of rTMS on the ability to process gestures, this series of studies has provided additional information in the search for greater clarity of the role of the motor cortex in language processing. Despite comparative extensions on methodology, the results presented here largely fail to replicate findings of previous researchers, such as the anticipated improvement of RT to hand related motor words when single-pulse TMS is applied at 90% RMT and 150 ms post-stimulus (Pulvermüller et al., 2005). The most remarkable exception to this is the duplication of results demonstrating and more negative N400 amplitude to incongruent speech-gesture pairings reported by Kelly et al. (2010). Aside from this, where the results do provide evidence, it largely contrasts with findings of previous researchers. Some of these may be explained by considering the methodological differences between this and previous research design. This is exemplified by the finding indicating a latency dependent acoustic 'click' effect of single-pulse TMS which was similar across TMS and control in Study 1, which draws into question Pascual-Leone et al.'s (1992) report on TMS latency effects in a simple RT task.

The widespread lack of significant findings reported throughout this thesis provide limited evidence for motor cortex involvement in language processing, and little support for the findings of previous authors who ascribe to this theory. Where significant findings are reported, they generally directly contrast with the theoretical

underpinnings intuited from the literature that pertain to the role of the motor cortex in processing linguistic information. One possible explanation for many of the results reported in this paper could be the notion that the motor cortex is not only involved in the processing of motor-related language, but may also be involved in other non-motor related linguistic processing. There are a number of findings that could be interpreted to support this theory . Firstly, as mentioned above, Pulvermüller et al. (2005) found that single pulse TMS over the hand area of the motor-cortex improved RT to hand related verbs compared to leg-related verbs. In empirical study one from this thesis, single-pulse TMS was applied to the hand-area of the motor cortex and RT was instead compared between non-action words and hand action word. The finding that TMS overall improved RT, with additional improvement seen for non-action words indicates that there is some general impact of motor cortex TMS on RT in a language processing task. That said, the finding that pulse latency affected RT regardless of whether the TMS was real or sham, indicates that there are other factors besides induced cortical stimulation (such as acoustic cueing) which may also have impacted RT in this case. Secondly, in the third empirical study it was found that where the application of cTBS did elicit variance in N400 amplitude, effects appeared in response to both speech-gesture and sentence stimuli. This again may be taken as an indication that alterations in motor-cortex excitability impact the processing of linguistic stimuli more broadly than action related language or gesture.

However, for these interpretations to be accepted as evidence that the motor cortex is involved in both motor and non-motor linguistic processing, they rely on a fairly restricted understanding of the impacts of a TMS-induced virtual lesion. Early interpretations of the impact of virtual lesions often ascribed to the notion that if a

specific cognitive function was interrupted following TMS of a targeted cortical region, this can be taken as evidence that the target region is somehow involved in that cortical function (Cohen et al., 1997). More recently published researchers have demonstrated, the effects of rTMS are thought to be much less focal than initially anticipated, and the 'reactive rapid plasticity' reported by authors such as Thiel et al. (2006) indicates that changes in brain activity are not just restricted to the lesioned area. As such, the above interpretations specifically refer to the impacts of TMS on the motor cortex and changes in motor cortex excitability without specifically ascribing the motor cortex with a *functional language processing* role. Regarding study three data, it was observed that there appeared to be right hemispheric changes in N400 latency following cTBS stimulation of the left hemisphere. If inter-hemispheric differences indicate change in distal (non-focal) cortical activity, this may be taken as evidence that there are compensatory cortical areas being engaged by motor cortex stimulation. Therefore, based on the current studies, it is impossible to determine whether it is the disruption of motor cortex function that impacts language processing, or whether these compensatory areas have some functional role in language processing that is being vicariously disrupted via cTBS of the motor cortex. That said, the fact that motor-cortex disruption causes changes in N400 amplitude at all is indicative that the motor cortex may be engaged by these tasks, otherwise it would be expected there would be no need for other cortical regions to compensate for a reduction in motor cortex excitability.

When considering the results from this study, it is also important to review recent evidence indicating there are limits to reliability of the cTBS protocol. Since cTBS parameters were first published by Huang et al. (2005), multiple researchers have employed cTBS as a method of reducing motor cortex excitability for an

extended period of time (e.g., Cárdenas-Morales, Nowak, Kammer, Wolf, Schönfeldt-Lecuona, 2010; Martin et al., 2006). Recently, Vernet et al. (in press) authored a paper in which they retrospectively examined excitability data from participants who received cTBS on two separate occasions. Vernet et al. reported high intra-individual variability between sessions, and they found that cTBS-induced depression in cortical excitability in one session, was not necessarily predictive of cTBS having the same effects in subsequent sessions. While Vernet et al. did observe cTBS induced changes in excitability in both sessions, the consistency of this depression across sessions was poor. Regarding the use of cTBS in the current studies, the choice to rigorously screen participants to only include individuals who showed a cTBS-induced *reduction* in excitability may have ameliorated some of these inconsistencies. However, without ongoing excitability checks during testing sessions (which were impractical due to the time-limited nature of cTBS effects), it is impossible to be certain that cTBS affected each individual consistently across sessions.

To clearly determine rTMS-induced cortical changes that may impact cortical functioning, experimental design must include more data collection methods that provide more direct and accurate evidence of brain activity. ERP results demonstrate specific temporal fluctuations in activity related to the processing of speech-gesture information, and therefore, understanding motor-cortex activity in processing linguistic information such as gesture requires a dataset that provides temporally accurate measurements of brain activity during specific interactions with stimuli. Further to this, the indication that there are widespread cortical effects of cTBS demonstrates the need for high resolution imaging data (such as fMRI) that can provide information on how activity in these brain regions vary over time. Ideally,

research in this area would also include imaging techniques that provide an indication of the way in which these brain areas engage within the context of a dynamic network of cortical activity. Advanced MRI techniques such as diffuse tensor imaging have been shown to provide advanced insights into the relationship between cortical areas during cognitive processing (Schmahmann et al., 2007) as well as following a genuine brain lesion (Mac Donald et al., 2011). To conclude, beyond speculation on the effects of rTMS on the motor- networks the results outlined in this thesis indicate how unclear the functional role of the motor-cortex in language processing is, the limits to the interpretability of behavioural data, and the necessity for supportive imaging data in order to draw strong conclusions on the impacts of rTMS on cognitive functions.

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